FACTORS AFFECTING THE GERMINATION OF SCLEROTIA AND THE SUBSEQUENT DEVELOPMENT OF THE STROMATA OF *CLAVICEPS PURPUREA* (FRIES) TULASNE

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The undersigned hereby certify that they have read and recommend to the School of Graduate Studies for acceptance, a thesis entitled, "Factors Affecting the Germination of Sclerotia and the Subsequent Development of the Stromata of Claviceps purpurea (Fries) Tulasne", submitted by Margaret Sarah Gibson, B.Sc., in partial fulfilment of the requirements for the degree of Master of Science.

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Abstract

A determination of the effects of certain factors on the germination of sclerotia of Claviceps purpurea (Fries) Tulasne and the subsequent development of stromata from them was made in a series of experiments reported hereinafter. Sclerotia required a dormancy period before they would germinate, but the temperatures near freezing usually recommended for good germination were not found essential under all storage conditions. A variety of substrata were used successfully in the germination experiments. Within the temperature range of 10° ± 2° C. - 23° ± 2° C. and the moisture range of 1.43% - 16.12% (by wt.) the sclerotia germinated (on a sand substratum). Ultra-violet rays under the conditions employed had no effect on germination. The effect of water soaking depended on the temperature of the soaking; soaking with subsequent freezing decreased germination. Sclerotial pieces germinated readily and generally produced normal stromata. Sclerotia did not germinate as well at the surface of the soil as at various depths within it up to 6 in. Alternately drying and wetting sclerotia did not influence time nor percentage germination. A number of auxins, phenolic compounds, Orthocide 75, Spergon, mercuric chloride and hydrogen peroxide did not influence the germination of sclerotia in the manner used, while ammonium sulphate, ammonium hydroxide, Javex, Arasan, Ceresan M, formaldehyde, and a solution containing asparagine and maltose did. Three imperfect fungi, Gliocladium sp., Trichoderma sp., and Trichothecium roseum, added to sterile soil prevented the germination of sclerotia. Sclerotia partially

devoured by larvae of Acylomus sp. were still germinative. Stromata were positively phototropic and negatively geotropic. The lengths attained by the stromata depended on the temperature and the light intensity to which they were subjected. A range in sand moisture from 4.58% to 14.74% (by wt.) did not affect the growth of the stromata. Stromata whose heads were removed regenerated new ones. For every capitulum removed a number were regenerated.

THE UNIVERSITY OF ALBERTA

FACTORS AFFECTING THE GERMINATION OF SCLEROTIA AND THE SUBSEQUENT DEVELOPMENT OF THE STROMATA OF CLAVICEPS PURPUREA (FRIES) TULASNE

A DISSERTATION
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Table of Contents

List of Tables

List of Figures

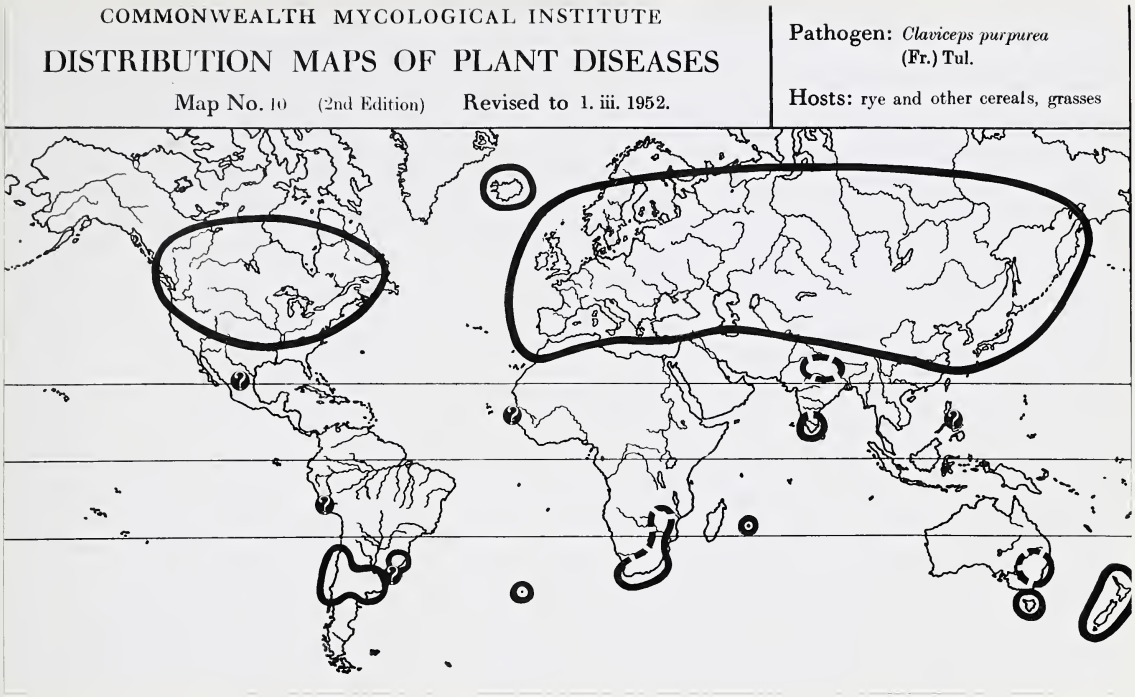

Fig. 1. Geographical distribution of Claviceps purpurea (Fries) Tulasne (copied).

Fig. 2. A germinated sclerotium of Claviceps purpurea (Fries) Tulasne with 146 stromata (original).

I. INTRODUCTION

The Ascomycete Claviceps purpurea (Fries) Tulasne is the causal agent of the ergot disease affecting rye, wheat, barley, oats, and a number of cultivated and wild grasses. This pathogen is very important both economically and historically.

The fungus causes a disease in animals and man called ergotism. This disease is caused by the poisonous alkaloids found in the sclerotia - the hard, dormant mycelial masses - which are eaten with grain or preparations from grain such as bread. Serious outbreaks since 857 A.D. have occurred in France, Germany, Russia, and the Scandinavian countries, according to Barger (4). As recently as August, 1951 four people died and two hundred were extremely ill in southern France after eating bread made from ergoty flour (1). Numerous reports of ergotism in livestock are found in literature.

C. purpurea is also beneficial. Alkaloids obtained from sclerotia have been used for quickening childbirth for many centuries (4). Youngken (44) has reported more recent uses of the alkaloids and derivatives in treating migraine headaches and decreasing hypertension.

Grain growers are very much concerned about the parasitic growth of C. purpurea, especially in rye and to a lesser extent in wheat and barley. Crop losses result from sclerotia replacing kernels in many heads. Also, a number of grains have been found

partially ergotized (13). Brentzel (6) reported a shrivelling of the non-ergotized seeds and a destruction of embryos by the fungus, without the development of sclerotia. Seymour and McFarland (38) found 47% of the florets held blasted kernels or were empty in 730 ergotized spikes. Of 651 unergotized spikes only 31% of the florets held blasted kernels or were empty.

Shortage of supply of sclerotia for pharmaceutical preparations has induced workers to cultivate the fungus in the field in the U.S.A. and Canada. However, this practice has been discouraged because of the probability of the spread of the organism to other fields.

The general influence of the environment on the development of the ergot disease is well known. Rojdestvensky, as reviewed by Barger (4), believed a rainy spring followed by a dry, sunny interval just before and at the beginning of the flowering period of rye and rainy weather during the rest of the growing season, suitable for the development of the ergoty rye.

C. purpurea has a wide geographical distribution, (fig. 1). The organism is not indigenous to Australia and New Zealand (4). It is believed to have been imported there with grain.

Life Cycle of *Claviceps purpurea* (Fries) Tulasne

Sclerotia fall to the ground from the cereal heads at harvest time or before (fig. 3,A). Normally they lie on the ground in a dormant state until the following spring. At this time if favourable conditions prevail germination occurs. Small white to yellow spherical heads emerge through the ruptured sclerotial surface. The number of heads per sclerotium may vary considerably. Generally there are between two and sixty (Figure 2 shows a sclerotium with 146 heads.). As growth continues the heads are pushed up by the stalks which bear them. The stalk or stipe and the head together are known as a stroma, (fig. 3, B). As the heads develop, cavities begin to form in them just below the surface. From the bases of the cavities arise ascogonia, each of which has a branch becoming an antheridium (20), (fig. 3, C). The walls dissolve between the ascogonium and antheridial arm and the male nuclei migrate into the female organ, (fig. 3, D). The nuclei pair. Ascogenous hyphae and ascal hooks are formed, (fig. 3, E). From the latter grow out the asci. While the asci are developing, thin perithecial walls develop around the sexual organs within the stromatal heads. As the perithecia approach maturity, each cylindrical ascus within develops eight, filamentous ascospores, (fig. 3, F, G). At maturity the ascal cap is pushed off the ascus and the ascospores are expelled forcibly into the air or more slowly just as far as the ostiole. In the former case convection currents aid in dispersion, while in the latter insects effect the distribution.

An ascospore upon landing on the moist stigma of a cereal or grass flower begins to germinate within 24 hours, (fig. 3, H). According to Kirchoff (22) and Ramstad (32) the hyphae do not enter the ovary via the interior of the style. The growth is down the outside of the style and the entry is at the base of the ovary. The ovary is detached from the receptacle and, for some unknown reason, the wound fails to heal. The plant sap exudes out. This secretion serves as a nutrient for the fungus. Soon the fungus begins to produce tiny, hyaline, oval conidia (1.5 - 2.5 μ in length in honey dew), (fig. 3, I). The sweet, yellow secretion containing the conidia, conidiophores, and hyphae is called the honey dew or sphacelial stage. Secondary infection may then occur following the dripping of the honey dew from upper flowers to lower ones. However, the dissemination is chiefly by various insects that feed on the honey dew. When the honey dew is diluted, for example by rain, the conidia germinate and new infections are initiated in the flowers, (fig. 3, J and K).

After the young ovary has been destroyed by the mycelium, this fungal mass with irregular cavities is gradually transformed from the lower end up. New branches of hyphae wind themselves between the original ones. All hyphae become septate and increase in thickness so that by mutual pressure a pseudo-parenchyma is formed. This is the beginning of the sclerotium. Gradually honey dew and conidial production ceases and concurrently a thin cortex is formed consisting of closely packed parallel hyphae which are normally violet to black in colour. The apex of the sclerotium is

often crowned with remnants of the ovary and stamens covered with the sticky honey dew. The sclerotia mature at a rate corresponding to that of the normal kernels.

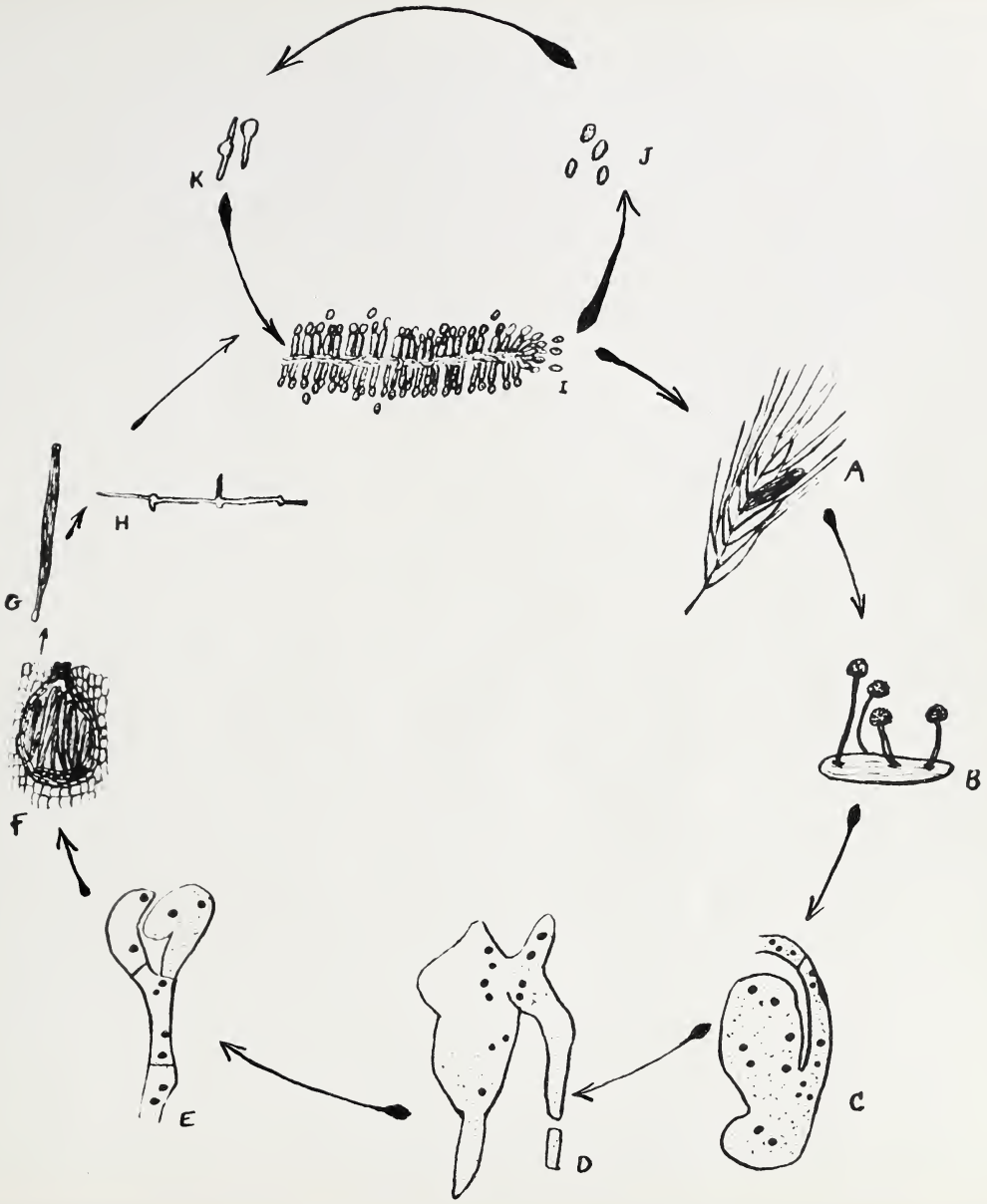

Figure 3. Life cycle of Claviceps purpurea (Fries) Tulasne. (After Alexopoulos).

A. Mature sclerotium on mature cereal head.
B. Sclerotium with mature stromata.
C. Archegonium with antheridial arm.
D. Plasmogamy.
E. Ascogenous hyphae and crozier.
F. Longitudinal section of perithecium.
G. Ascus.
H. Germinated ascospore.
I. Mycelial mat, conidiophores, and conidia.
J. Conidia.
K. Germinated conidia.

The Taxonomical Affinities of *Claviceps purpurea* (Fries) Tulasne

Claviceps is generally placed in the Hypocreales. The members of this order are distinguished by brightly colored ostiolate perithecia with relatively soft, waxy walls. Since color and consistency of the stroma or perithecium are not considered satisfactory as a basis for distinction between the Sphaeriales and Hypocreales, Miller (27) uses internal perithecial characters. He, therefore, places all Clavicipitaceae in the Sphaeriales. The fungi in this order have asci within a wall layer of apically free paraphyses.

All members in the family Clavicipitaceae have a well-marked cap at the apex of the long cylindric ascus, and within the ascus are filiform spores which break up into segments at maturity. The ascal cap is perforated through the centre by a very narrow pore through which the spores are not extruded. Rather, the entire cap is pushed off and the spores are expelled. The asci arise from a basal plectenchyma and never lie in the wall layer as in members of the other families. Paraphyses and periphyses are formed in most species but deliquesce early.

Barger (4) gives a detailed account of Stäger's results which showed races of *C. purpurea*. Stäger's work has more recently been severely criticized by a number of workers. Békésy (5) found that *Bromus erectus*, *Lolium perenne*, and *Melica uniflora* could be infected by using inoculum from rye. Stäger had reported

the opposite. Campbell (8) found 283 isolates of C. purpurea from various grasses and grains were equally effective in causing ergot in rye, wheat, and barley. It is now believed Stäger's work was inadequate to justify the divisions proposed.

Grasso (13) carried out a careful study of C. purpurea, C. microcephala, C. sesleriae, and C. setulosa. All results, including those from inoculation experiments, made him conclude that the latter three are synonymous with C. purpurea.

The Scope and Purpose of the Present Study

The present work was confined to the dormant and post dormant stage of the fungus. This, therefore, included a study of the germination of the sclerotia and the development of stromata by germinated sclerotia. The dormant or sclerotial stage is an obligatory part of the life cycle of the fungus. If the sclerotia do not germinate in the spring or early summer, new infections will not occur in the current cereal crops and grasses. By breaking the life cycle at this stage it would be possible to eliminate the fungus because the conidial stage is not known to overwinter. Therefore, factors physical, chemical, and biological that influence the germination of the sclerotia were studied, as well as physical agents affecting the development of the stromata.

The purpose of the present study was to learn more about the germination and development of the stromata, with the ultimate aim of determining a practical way (or ways) of interfering with the life cycle at these stages so that it may not go to completion.

II. Germination of Sclerotia

Introduction

In 1852 Tulasne showed that the sclerotium is just a dormant stage of the fungus. He found the ergot bodies will germinate with the production of stromata. For many years after this the belief that the sclerotia are difficult bodies to germinate was retained. As the following experiments will show, provided the right conditions prevail, the sclerotia will germinate well.

The environment plays no small part in the germination of sclerotia. Long rainy spells in France in 1951 (1) were conducive to germinating the sclerotia. Generally grasses in low, damp spots and along streams have been found more severely ergotized (2). Markhasseva's study (26) in the U.S.S.R. during 1935 made her conclude that sclerotia germinate under natural conditions after a rest period of 8 - 9 months. Germination was found best if the soil moisture was about 22% and the temperature was 10° C. or higher.

Some textbooks state that sclerotia will not germinate after they are one year old. More recent workers have shown that sclerotia one or more years old are still germinative (6, 17, 41). Because of lack of material, no research work was done in this direction.

More detailed discussions regarding the other factors are given in the sections which follow.

Experimental Methods for the Germination of Sclerotia

Clean petri dishes either 3/4 in. or 15/16 in. deep and clay porous pots 6 in. diameter were the two types of containers employed for the germination of sclerotia. Black soil and white sand from the greenhouse and white blotting paper were the chief substrata employed. Walk-in coolers as well as ice-box size coolers were used for incubating the sclerotia at the various temperatures indicated in the experiments.

To 50 gm. soil per petri dish was added 20 ml. distilled water; for 50 gm. white sand 10 ml. distilled water was used. Sterile soil was prepared by placing the soil in the petri dishes and sterilizing for 3 hr. at 15 lb. pressure. The blotting paper was cut into disks of just slightly less diameter than the inside of the petri dish bottom. Blotting paper in the petri dishes was sterilized by leaving the dishes overnight in the hot air oven at 250° F. Just before using, 3 ml. sterile, distilled water was added to each dish containing the blotting paper by means of a sterile pipette. If sclerotia were sterilized in any manner, forceps sterilized by dipping in alcohol and then flaming were employed in the transferring.

The auxins employed were put into solution by dissolving 0.1 gm. in 2 ml. absolute alcohol. The solution was added to about 75 ml. boiling water; boiling was continued for 5 min. The solution was then cooled, poured into 100 ml. volumetric flask

and more distilled water was added to make 100 ml. The 1000 p.p.m. solution thus made was diluted with distilled water to the desired concentration.

The cornmeal-sand medium on which the various fungi were grown for the experiment determining their effect on germination of sclerotia was prepared by mixing 1000 gm. sand, 200 gm. cornmeal, and 250 ml. H_2O. About equal amounts of the mixture were placed in 500 ml. flasks. The flasks were plugged with cotton and placed in the sterilizer for 3 hr. at 15 lb. pressure. The fungi were transferred from potato dextrose agar slants in test tubes to the cornmeal-sand mixture where they were allowed to grow for one week. The flasks were shaken daily for one hour. Small, nearly equal amounts of each fungus and medium were transferred by sterile forceps to the sterile soil in the petri dishes.

The hanging drops used in the germination of ascospores were prepared by placing three drops of sterile, distilled water with the ascospores on the inside of a lid of a petri dish. The lid was placed over the bottom which contained moist blotting paper.

The Dormant Period Requirement for the Germination of Sclerotia

Most authorities agree that sclerotia remain in a dormant state for a period of time after their formation. While freezing temperatures were at one time believed most conducive to breaking the dormancy, it is now believed exposure to temperatures near freezing is just as effective. MacFarland (24) found the shortest

period of rest for the sclerotia to be 8 weeks. Kirchoff (22) stated exposure to 2° - 3° C. during 3 - 6 weeks resulted in 60 - 80% germination after 4 - 8 weeks at 15° C.; after an exposure to 8° - 10° C. only about 10% germinated. Duration of time in the cold he found important; after the short period of 15 - 20 days in the cold a few sclerotia germinated but 7 - 9 weeks were required at 14° - 16° C. Brown (7) germinated sclerotia within a month at room temperature after they had been 2 months in stratified, moist sand at 5° C.

Experimental:

Sclerotia were collected from rye August 15 - 17, 1955. After remaining at room temperature until August 24 one third the total number was placed in the cooler (10° C.). The remainder were put in screen bags and buried about one inch below the surface of the soil in a cultivated field. On October 11 sclerotia were gathered from beneath rye plants where they had fallen about three weeks previously. The latter sclerotia were planted immediately in black soil in petri dishes and placed in the cooler (15° - 18° C.) for germination. On October 7, 1955 some of the sclerotia were brought in from storage in the black soil, planted in black soil in petri dishes and placed in the cooler (15° - 18° C.). Sclerotia were taken from the cooler September 2, 1955, planted in black soil in petri dishes, and returned to the cooler (10° C.).

Table 1 shows the germination results for these sclerotia. Clearly a dormant period was favourable for the germination of

Table 1. Dormant period requirement for germination of sclerotia

Date removed from storage	Place and ave. temp. of storage	Time stored	Temp. for germination	No. sclerotia germinated / No. sclerotia planted (Oct. 27)
September 2, 1955	Cooler, 10° C.	10 days	10° C.	3/20
September 2, 1955	Cooler, 10° C.	10 days	20°-22° C.	0/20
October 7, 1955	Black soil, 9° C.	37 days	15°-18° C.	18/38
October 11, 1955	Beneath rye plants, 9° C.	20 - 25 days	15°-18° C.	3/15

sclerotia. Ten days at 10° C. resulted in very low germination over one month after the planting time, while exposure to about 9° C. for 37 days gave 47.4% germination after 20 days. However, the fluctuating temperatures out-of-dours (There were two nights of frost.) and the exposure of the sclerotia to the soil rather than the atmosphere of the cooler probably influenced the results. In any case the sclerotia germinated fairly well without the prolonged exposure to temperatures of 2° - 3° C. deemed necessary by Kirchoff. Since sclerotia germinate readily at temperatures from 10° to 22° C. (Later experiments showed this.) the actual germination temperatures used would not be expected to affect the results greatly.

In another experiment on dormancy the sclerotia were gathered from Prolific rye plants in the greenhouse on June 20, 1956. These ergot bodies were planted immediately on moist, sterile blotting paper in petri dishes. The dishes were placed in the cooler

(10° C.). Not until October 15, 1956 did any germination occur. By October 22, 10 of the 18 sclerotia planted had germinated.

The Effect of Different Substrata on the Germination of Sclerotia

Moist sand has been employed by many workers (7, 30, 37, 43) as the substratum for sclerotial germination. Brentzel (6) found moist sandy soil a good substratum, while on potato dextrose agar or moist, filter paper in petri dishes he did not have any sclerotia germinate. Henson and Valleau (19) found 1% water agar in cotton-plugged vials a most useful medium for germination because no watering or any other treatment was required during the period the cultures were subjected to conditions for germination.

A. Different Soil Types and the Germination of Ergot Bodies

Each soil type with its particular physical and chemical make-up and microflora would be expected to have some effect on the germination of sclerotia. Indeed, Pammel, as reported by Atanasoff (2), found that ergot bodies flourished very well on rich soil.

Experimental:

Three fresh samples of brown soil from different locations in Southern Alberta, Edmonton black soil, grey soil from Breton, and washed, white sand were tested for their effects on sclerotial germination.

Deep petri dishes partially filled with 50 gm. of each soil type were used. The soil in each dish was moistened with 20 ml. of distilled water, except for the dishes of sand where 10 ml. per dish was added. Four dishes with 6 sclerotia per dish were used for each soil type. The sclerotia tested were taken from the field where they had been stored since the previous September. Incubation was at 20° - 22° C. The experiment was started November 3, 1955, and final results were taken December 12, 1955.

Germination had started in all dishes, except those containing sand, by November 14. By November 21 sclerotia in the sand also began to germinate.

Results in table 2 show that number of sclerotia germinated in the four dishes for each type of soil was very different.

Table 2. Effect of different soils on germination of sclerotia

Soil Type	Number of sclerotia germinated out of six planted per dish			
	Plate 1	Plate 2	Plate 3	Plate 4
Brown soil* (wheat field)	4	1	0	2
Brown soil* (barley field)	0	3	4	1
Brown soil (South Granum)	1	0	4	2
Black soil	5	0	2	0
Grey soil	2	5	4	5
White sand	3	0	5	1

* Soils from Picture Butte

However, between the different soil types there was not a great difference in germination, with the exception of the grey soil type. A comparison of the number of sclerotia germinated in the latter soil with that in the Edmonton black soil by the t - test showed a significant difference at the 5% level.

The fungi from the soil types that grew on the sclerotia were different. Trichoderma sp. was most prevalent on the brown soils and the white sand while Gliocladium sp. was common on the sclerotia on the black and grey soils.

In another experiment designed to obtain information on the significance of soil reaction on the germination of sclerotia greenhouse soil, white sand, and six old soil samples from B.C. were compared. The different soils chosen varied in pH at the beginning of the experiment from 4.6 to 7.9 as determined by the Beckman potentiometer. Each determination was made from a thick paste of soil mixed with distilled water. Six sclerotia were planted in each petri dish containing 50 gm. soil moistened with 20 ml. distilled water. Two dishes rather than 4 were used for each soil type because the soil samples on hand were small. Sclerotia used were from the lot stored on the soil. The experiment was started November 7, 1955 and final results were taken December 12, 1955. Incubation was at $15^{\circ} \pm 1^{\circ}$ C.

By November 21 germination had occurred on all plates where it did ultimately occur, except on the most acid clay loam and muck soils. Germination of sclerotia on these plates occurred one week later.

Final germination results from this experiment are shown in table 3. Since the subsamples were of necessity very small, the

Table 3. Effect of different B.C. soils on germination of sclerotia

Kind of soil	pH of soil at beginning	Number of sclerotia germinated out of six planted per dish	
		Dish 1	Dish 2
Clay loam	4.6	1	0
Muck	4.7	2	1
Alberta greenhouse (black)	5.5	1	2
Loam 47A	5.5	3	3
Loam 48	5.6	1	2
White sand	7.3	3	3
Pit	7.5	2	2
Kamloops	7.9	2	0

(pH of all soils at end of experiment was 7.0 - 8.0.)

fact that the sclerotia did not germinate as soon in the relatively acid soil or that the germination was lowest in the clay loam does not necessarily mean that the initially low pH values were unfavourable for germination.

In this connection a subsequent experiment designed for determining the effect of different pH values of buffered solutions on the germination of sclerotia is pertinent. Ergot bodies that had

been stored in the field all winter were soaked 3.75 hr. in phosphate buffered solutions whose pH values ranged from 4.1 to 8.1. The sclerotia were then planted on washed white sand that was moistened with the buffered solutions. The germination values after 1 month incubation along with the initial pH values were: pH 4.1 : 1, pH 5.1 : 2, pH 6.9 : 6, pH 8.0 : 0, pH 8.1 : 2. After 2 months the final pH values were found to range from 7.4 to 8.2 for all the dishes. By this time the respective germination values for the above pH values were: 5, 7, 10, 3, and 5. (For each buffered solution 24 sclerotia were used.)

These results suggest the relatively high or low hydrogen ion concentrations to which the sclerotia were subjected at the beginning might have been influential in delaying germination. Germination of sclerotia that had been initially subjected to pH 6.9 was slightly better than that of the checks where the sclerotia had been soaked in sterile water (pH 6.8) and then planted on moist sand.

It is uncertain why the pH values of the soil samples as well as the sand moistened with the buffered solutions should have become generally 7 - 8 after the incubation period. In dishes where sclerotia did not germinate the pH values were still about the same as the others. Either the bacterial flora on the sclerotia was instrumental in bringing about the change and/or basic substances pass out of the sclerotia. If it is the latter case, the passage must be fairly slow because sclerotia soaked for one week in distilled water did not change the pH of the water (pH determined colorimetrically).

B. Use of Sterile, Moist Blotting Paper in Petri Dishes as a Medium for the Germination of Sclerotia

Although Brentzel (6) found sterile, moist filter paper in petri dishes an unsatisfactory medium for the germination of sclerotia, white, moist, sterile blotting paper was found quite useful in the present studies.

The percentage germination was just as good as on the soils (about 60%), while the growth of contaminants was much less. The time sclerotia had to be subjected to germination temperatures was 1 - 2 months. Since cellulose is a relatively inactive substratum, various chemical solutions were substituted for water when it was desired to determine their effects on the germination of sclerotia. The chief disadvantage in using blotting paper was the tendency for the cultures to dry out rather rapidly in the coolers. Generally 1 - 2 ml. water had to be added to the cultures every week.

The Effect of Moisture Content of the Substratum on the Germination of Sclerotia

Most workers (22, 37, 43) have vaguely stated that the soil in which the sclerotia are germinated must be moist. As previously mentioned Markhasseva (26) found 22% moisture content of the soil favourable for sclerotial germination while Henry (17) has reported only 7% of the sclerotia germinated in soil with 10% moisture and no germination took place in wet or saturated soil.

Experimental:

White sand, thoroughly washed with tap and then distilled water before drying in the hot air oven, was used as the substratum. In each deep petri dish 50 gm. soil was placed. To each of two petri dishes the following quantities of sterile, distilled water were added: 3 ml., 4 ml., 5 ml., 7 ml., 9 ml., 10 ml., 12 ml., 15 ml. Six sclerotia were planted in each dish. The dishes containing the moist sand and sclerotia were weighed at the beginning of the experiment and weekly thereafter. Sterile, distilled water was added when necessary to bring the weight back to the original. Dishes were stored in a cooler at 15° C. The experiment was started May 15, 1956 and final results were taken June 27, 1956. Sclerotia had been stored in the field from September, 1955 until used.

At the end of the germination period the sclerotia were removed from the sand. The sand from each dish was quickly divided into two portions and each amount was placed in a clean, aluminium weighing dish that had previously been dried to constant weight. The weight of each dish containing the moist sand was determined before the dishes were placed in the drying oven (250° F.) overnight. The weights of the dishes containing the dried sand were then determined. Percentage moisture of the sand by weight was calculated.

The results are recorded in table 4. Clearly, the data show there was a range of percentage moisture content of sand in

Table 4. Effect of moisture content of sand on germination of sclerotia

% Moisture of sand (by wt.)	% Germination (6 sclerotia per dish)	% Moisture of sand (by wt.)	% Germination (6 sclerotia per dish)
0.03	0	10.27	33.3
0.38	0	10.80	83.3
0.48	0	12.94	66.7
1.43	16.7	13.02	33.3
2.19	33.3	13.72	83.3
2.76	16.7	14.78	66.7
5.34	66.7	16.12	50.0*
5.87	33.3	18.64	0
7.91	50.0		

* No germination occurred until 37 days after beginning of incubation. No further development was seen by June 27, 1956.

which sclerotia would germinate. However, in very wet sand (16.12% moisture by wt.) germination was very tardy and further development was slow.

In a similar manner the percentage moisture of black soil in which germination of sclerotia was good was determined. Values 23 - 24% moisture (by wt.) were obtained. Higher values would be expected for soil than for sand because there would be a greater availability of water in the sand.

The Effect of Temperature on Germination

Kirchoff (22) found 60 - 80% of the sclerotia germinated at 15° C. Minimum temperature he gave for germination was slightly above 10° C. and the optimum lay between 18° and 22° C. Schweizer (37) had sclerotia germinate well at 15° - 20° C. After the sclerotia were incubated at room temperature for a month, Brown (7) achieved good germination. Henry's work (17) showed that germination occurred at 10° - 20° C. while none occurred at 5° C. or 25° C.

Experimental:

Sclerotia that had overwintered in the field from September, 1955 until May 15, 1956 were used. Moist, white sand was the substratum on which the sclerotia were planted. Previous to planting, half the total number of sclerotia was surface sterilized in 2% Javex solution for 3 min. and then washed with sterile, distilled water. Dishes and sclerotia were weighed at the beginning of the experiment and water was added whenever necessary so that the moisture content was about the same in all dishes. Three dishes of treated sclerotia and three of untreated sclerotia were put at the following temperatures: 3° ± 2° C., 10° ± 2° C., 15.6° C., 15° ± 2° C., 20° ± 2° C., 23° ± 2° C. The experiment was started May 30, 1956 and final results were recorded June 29.

The results are given in table 5. No data are given for the dishes incubated at 20° ± 2° C. because the sclerotia in these

Table 5. Effect of temperature on germination of sclerotia

Temp. (° C.)	Untreated Sclerotia			Surface Sterilized Sclerotia*		
	No. of sclerotia germinated per dish (6 planted)			No. of sclerotia germinated per dish (6 planted)		
	Dish 1	Dish 2	Dish 3	Dish 1	Dish 2	Dish 3
3° ± 2°	0	0	0	0	0	0
10° ± 2°	6	6	5	2	0	0
15.6°	5	6	5	4	6	5
15° ± 2°	6	5	6	5	4	3
23° ± 2°	0	1	0	0	1	3

* Sclerotia surface sterilized with 2% Javex solution.

dishes became severely contaminated and had to be discarded. However, the experimental results given in table 2 are from sclerotia kept at 20°- 22° C. Table 5 does show less germination of sclerotia that were surface sterilized with 2% Javex solution. By use of the t - test no significant difference was found between untreated and surface sterilized sclerotia at the 5% level.

Sclerotia which did not germinate at 3° ± 2° C. germinated readily when placed at 15° ± 2° C.

The Effect of Soaking in Water on the Germination of Sclerotia

While no experimental results have been reported concerning the effect of soaking in water on the germination of C. purpurea

sclerotia, some have been published concerning sclerotia of other fungi. King et al.(21) found that sclerotia of Phymatotrichum omnivorum (Shear) Duggar would germinate (i.e., would produce hyphae) after being soaked in distilled water containing some sand for three months at room temperature. Only after 121 days did many show signs of disintegration. Moore (28) studied the effect of flooding different types of soil in Florida on the ability of sclerotia of Sclerotinia sclerotiorum (Lib.) D By. to survive. In flooded marl, muck, and sandy soils the sclerotia decayed within 23 to 45 days in the laboratory and in field tests. Moore decided that the microflorae of the various soils influenced the rate of decay.

Experimental:

A preliminary experiment to determine the effect of soaking on the germination of sclerotia was carried out during November and December, 1955. Sclerotia, collected in October and stored in a cooler (5° C.), were used. After the sclerotia were soaked at 10° C. in water from snow for 1, 3, 5, or 7 days they were placed on black soil moistened with snow water in petri dishes. The dishes were incubated at 20° C. In other cases after the soaking for 3, 5, or 7 days the sclerotia were put in vials and placed outside where they were frozen. Those soaked 3 or 5 days were frozen 2 days while those soaked 7 days were frozen 9 days at temperatures ranging approximately from -5° C. to -20° C. After freezing the sclerotia were tested for germination in the manner

described above. Sclerotia neither soaked nor frozen were also planted in black soil to serve as checks.

In all cases there was some germination but it was very poor, except where the sclerotia were neither soaked nor frozen. The two experiments which follow deal with the effects of soaking and freezing in more detail. In this preliminary experiment it was observed that various fungi and bacteria had formed luxuriant colonies on the treated sclerotia.

The Effect on Germination of Soaking Sclerotia in Water at Relatively Low Temperatures

Experimental:

Into each of several erlenmyer flasks containing 100 ml. sterile, distilled water per flask 20 sclerotia were placed. Half of the total number of sclerotia used was surface sterilized in 2% Javex solution for 4 min. After washing them in sterile, distilled water they were placed in the erlenmyer flasks for soaking. Soaking was continued for 89 hr. at the following temperatures: 3° - 4° C., 17° C., $24^\circ \pm 2^\circ$ C. Ninety sclerotia were soaked at each temperature; of these, 45 had been surface sterilized with 2% Javex solution. Checks consisted of 25 sclerotia neither surface sterilized nor soaked. Sclerotia were planted (5 per dish) on moist sand in petri dishes and incubated at 15° C. From September until May the sclerotia had been stored in the field. After this time they were kept at 5° C. until used (June 5, 1956). Final results were taken July 29, 1956.

The results are given in table 6. Clearly, soaking sclerotia in water at about 24° C. did greatly reduce the germination compared with soaking at the lower temperatures listed, namely 17° C. and 3° - 4° C.

Table 6. Effect of soaking for 89 hours in water at different temperatures on germination of sclerotia

Temp. of Soaking (° C.)	Untreated Sclerotia No. of sclerotia germinated per dish (5 planted)									Surface Sterilized Sclerotia No. of sclerotia germinated per dish (5 planted)								
	D1*	D2	D3	D4	D5	D6	D7	D8	D9	D1	D2	D3	D4	D5	D6	D7	D8	D9
3° - 4°C.	5	5	5	5	4	3	3	2	0	5	5	3	1	0	3	4	0	1
17° C.	4	3	4	1	4	3	4	5	1	2	2	1	2	0	2	1	1	2
24° ± 2°C.	1	0	0	0	2	1	1	0	2	0	0	0	0	0	0	0	0	0

* D1 = Petri dish 1

Although sterile, distilled water was used for the soaking and many sclerotia were surface sterilized with 2% Javex, there was still bacterial growth in all soaking water. It is very likely that the higher temperature was more favourable for bacterial growth. This in turn possibly had an adverse effect on the ability of the sclerotia to germinate. Table 6 also shows that sclerotia surface sterilized in 2% Javex solution before soaking germinated less well in comparison to those not treated. Comparison of the germination of treated and untreated sclerotia by the t - test gave a significant difference at the 1% level. Therefore, under the conditions

employed, surface sterilization with 2% Javex solution for 4 min. did decrease the germination of the sclerotia significantly.

Of the sclerotia neither soaked nor surface sterilized 40% germinated.

In no case were fungi seen growing on the sclerotia in the sand. This was a marked contrast to the prolific growths found in all dishes in the preliminary experiment where the sclerotia had been soaked in snow water and then planted on soil.

The Effect of Soaking Followed by Freezing on the Germination of Sclerotia

Since the previous experiment indicated that soaking for 89 hr. in relatively pure water had no detrimental effect on the germination of sclerotia, an experiment was set up to determine primarily what effect freezing after soaking would have on the germination of sclerotia.

Experimental:

Sclerotia, collected in September, 1956 from Prolific rye and stored in a cooler (2° - 5° C.), were soaked in sterile, distilled water for 4 days at 15° C. Of the 400 sclerotia soaked in this manner 200 were previously surface sterilized with 1% aqueous phenol solution for 3 min. After the soaking, 200 of the sclerotia were frozen (-10° C.) for one week in petri dishes on greenhouse soil or moist blotting paper. A check consisting of 50 untreated sclerotia planted on moist blotting paper was used.

The sclerotia that were only soaked were planted on greenhouse soil or sterile blotting paper. Ten sclerotia were planted in each dish. The experiment was started November 24, 1956 and final results were taken February 1, 1957.

Table 7. Effect of soaking in water followed by freezing on germination of sclerotia

Treatment	No. Sclerotia Germinated on Blotting Paper (10 planted per dish)					No. Sclerotia Germinated on Soil (10 planted per dish)				
	D1*	D2	D3	D4	D5	D1	D2	D3	D4	D5
Soaked 4 days in sterile distilled water	8	6	7	5	8	3	2	2	2	6
Surface sterilized sclerotia soaked 4 days in sterile, distilled water**	9	9	10	6	10	7	6	4	4	3
Untreated sclerotia frozen (-10° C.) for 1 week after soaking (as above)	0	0	0	0	0	1	0	1	7	1
Surface sterilized sclerotia frozen (-10° C.) for 1 week after soaking (as above)	0	0	0	9	2	0	0	1	2	1
Check - no freezing or soaking	1	7	10	9	9	-	-	-	-	-

* D1 = Petri dish 1
** Sclerotia were surface sterilized with 1% aqueous phenol for 3 min.

Final results are shown in table 7. The germination of the sclerotia frozen for 1 week was considerably lower than that of those soaked for 4 days. The soaking for 4 days did not affect the germination. Microorganisms in the greenhouse soil were probably

influential in reducing germination on that medium in comparison with that on sterile, blotting paper. At least bacterial and fungal colonies were very evident on the sclerotia and the soil surrounding them.

Another 60 weighed sclerotia were soaked 4 days in sterile, distilled water. After these sclerotia were used to determine the gain in weight due to the absorption of water, they were wrapped in filter paper and also frozen for a week (-10° C.). This test was run concurrently with the experiment described above.

The sclerotia that had doubled their weights by soaking in water 4 days had also low germination (20%).

The Effect of Ultra-violet Radiation on the Germination of Sclerotia

The effects of ultra-violet rays on vegetative growth of C. purpurea on an agar medium was studied by McCrae (25). Use of ultra-violet rays (4.3 amp., 150 volts, 21 cm.) for periods of 5, 10, or 15 minutes did not kill the cultures. Gröger (14) found ultra-violet radiations produced mutants which had white sclerotia, while spontaneous mutations seldom occurred for this characteristic.

Apparently no studies on the effects of ultra-violet rays on the production of the perfect stage of C. purpurea have been made. However, many investigators have studied the effects of ultra-violet rays on the sporulation of other fungi. Stevens (39) showed that ultra-violet radiation induced the formation of perithecia by various isolates of Glomerella cingulata a few days

after irradiation. Remsberg (33) found the sclerotia of Typhula sp. only after exposure to ultra-violet light would readily produce the perfect stage of the fungus.

Experimental:

In the first experiment performed the effect of ultra-violet light on the germination and subsequent development of stromata was determined. The irradiation for 10 min. was given just when germination was beginning for some of the sclerotia in all of the dishes. Treatment was from a 120 volt, 15 watt lamp at a distance of 20 cm.

Twenty plates, 10 containing white sand and 10 others partially filled with greenhouse soil, were used. In each dish 5 sclerotia were planted. The sclerotia had overwintered on the soil. The dishes were placed in a cooler ($15^{\circ} \pm 2^{\circ}C.$) May 10, 1956 and on May 29 when some of the sclerotia were beginning to germinate the dishes were removed from the cooler. Ultra-violet radiation treatment was given to the sclerotia in 10 of the dishes (lids removed). The other 10 dishes served as checks. The dishes were returned to the cooler until June 29, 1956 when the final results were recorded.

The ultra-violet radiation given did not visibly affect the germination or subsequent development of the stromata of these sclerotia. The final germination results are given in table 8.

Table 8. Effect of ultra-violet radiation on germination of sclerotia

Ultra-violet radiation treatment	Substratum	No. of sclerotia germinated per dish (5 planted)				
		Dish 1	Dish 2	Dish 3	Dish 4	Dish 5
10 min. at 20 cm.	Greenhouse soil	3	3	3	4	4
None	Greenhouse soil	2	3	5	4	3
10 min. at 20 cm.	White sand	1	2	0*	4	4
None	White sand	0*	3	0*	0*	3

* On these plates Rhizopus sp., a contaminant, was covering the sclerotia

Another experiment was carried out using ultra-violet radiation on sclerotia more than once. After the sclerotia were planted they were given a 10 min. treatment (at the same distance and with the same lamp as in the previous experiment). Every week thereafter until the final results were taken they were given another 10 min. irradiation.

Twelve petri dishes containing sterile, blotting paper were used. Six sclerotia were placed in each dish, after being surface sterilized with 2% Javex solution and washed in sterile, distilled water. The sclerotia had been kept in the field from September until May and then in a cooler (2° - 3° C.) until used (June 20, 1956). Sclerotia in 6 dishes were given the irradiations. The six untreated dishes were taken from the cooler every time the other 6 were irradiated. Final results were recorded July 31, 1956.

Table 9. Effect of ultra-violet radiation on germination of sclerotia

Ultra-violet radiation treatment	No. of sclerotia germinated per dish (6 planted)					
	Dish 1	Dish 2	Dish 3	Dish 4	Dish 5	Dish 6
10 min. per week, 6 exposures	5	6	2	1	5	3
None	2	2	5	2	3	5

By referring to table 9 it is seen that there was no difference in the germination of irradiated and non-irradiated sclerotia.

The development of the stromata was normal.

The Germination of Sclerotia Planted at Different Soil Depths

Brentzel (6) found a number of sclerotia sent stromatal heads up through an inch of soil, but those planted deeper had very few heads emerging at the surface. Henry et al.(18) found in field tests that the heads never reached the surface when the sclerotia were planted 2 in. deep.

Experimental:

This experiment was undertaken primarily to determine whether:

(a) The depth of planting has any effect on the germination of sclerotia.

(b) The heads of the stromata can reach the surface when the sclerotia are planted 2 in. deep in the soil.

In the clay pots containing moist greenhouse soil the sclerotia were planted on top of the soil, just beneath the surface of the soil, 2 in. deep, or 6 in. deep. Four pots were used for each depth of planting. In each pot 12 sclerotia were planted. The pots were placed in a cooler (15° - 16° C.) where the lights were on periodically. The sclerotia used in the experiment had overwintered out-of-doors in the soil. In May they were placed in a cooler (2° - 3° C.) and kept there until June 8, 1956 when the experiment was started. Final results were recorded July 11, 1956.

The viability of the ascospores produced in the stromatal perithecia from the sclerotia at all levels in the soil was determined by the hanging drop technique previously described.

Table 10. Germination of sclerotia planted at different soil depths

Depth of planting	No. of sclerotia germinated per pot (12 planted)			
	Pot A	Pot B	Pot C	Pot D
On the surface	3	2	2	1
Just beneath the surface	5	6	7	6
2 In.	11	9	7*	6*
6 In.	7	9	9	5

* In each of these two pots one stromatal head reached the surface.

Final germination results are given in table 10. The low germination values for the sclerotia planted at the surface of the soil are significantly different from the values for sclerotia at other levels (by t - test). The low germination may be attributed to a number of factors:

(a) The sclerotia exposed to the air would be drier than those completely surrounded by moist soil.

(b) Fungal growth occurred on portions of these sclerotia (no fungal growth on the others).

(c) The periodic light in the cooler may have had an adverse effect.

In each of 2 pots where the sclerotia were planted 2 in. deep 1 stromatal head reached the surface. That is, from 33 sclerotia germinated only 2 heads out of approximately 1500 reached the soil surface.

The stipes of the stromata produced by the sclerotia at the surface of the soil were much shorter than those produced by sclerotia at various depths in the soil. A later experiment showed that light inhibited linear growth of the stipes.

Viable ascospores were produced regardless of the depth of sclerotial planting.

The Effect on the Germination of Alternately Drying and Wetting Sclerotia

Klebahn, as reported by Barger (4), recommended the alternate drying and wetting of sclerotia for an acceleration in germination. The effect of such a treatment was compared with

that of continuous moisture in the following experiment.

Experimental:

Into each of two sterile petri dishes containing moist blotting paper were placed 20 sclerotia. The dishes were then weighed. Every other day the dish (with lid removed), designated for the drying treatment, was put into a desiccator that contained calcium chloride in the bottom. The dishes were reweighed periodically and sterile water was added when necessary to bring the weight back to the original. The wetting and drying process was carried on in a cooler for a month at 10° - 12° C, where the dishes were incubated throughout the experiment. During the following month no treatment was given to either dish. The experiment extended from December 3, 1956 to February 4, 1957 when the final results were recorded. The sclerotia used in this experiment had been collected from Prolific rye in September, 1956. They were stored at first in a cooler (5° C.); later they were put in a refrigerator (-10° C.) for 30 days.

The final results are shown in table 11. Plainly the alternate drying and wetting did not influence the germination of the sclerotia.

Table 11. Effect of alternately drying and wetting sclerotia on germination

Treatment	No. of sclerotia germinated (20 planted)	% Germination
Drying and wetting every other day for one month	14	70
Continuous moisture	15	75

In both dishes there were many stromata on each sclerotium, and, contrary to Klebahn's belief, there was no difference in the time required for germination. The stromata produced on the sclerotia in the dish receiving continuous moisture were slightly more advanced in development than those produced in the other dish.

Germination of Sclerotial Pieces

Köhler, as reported by Barger (4), found that sclerotial pieces would germinate. Brentzel (6) was unsuccessful when he endeavoured to germinate sclerotial pieces. MacFarland (24) found that the removal of the cuticle (Presumably he meant the black, thickened, outer cells.) from sclerotia with a scalpel does not prevent their germination. However, he found the stromata were always deformed and all seemed to arise from a stromatic cushion.

Experimental:

The sclerotia were softened by soaking them for 16 hr. in sterile, distilled water. Cross sections 0.3 - 0.5 cm. in

height, peelings about 1 mm. thick, longitudinal pillars of internal mycelium about the length of the sclerotia and 2 - 3 mm. in diameter, and sclerotial halves (cut longitudinally) were prepared. Whole sclerotia served as checks. The whole sclerotia or pieces were surface sterilized in 2% Javex solution about 3 min., washed in sterile, distilled water, and planted on a white sand substratum in petri dishes. Three petri dishes were used for pieces of sclerotia cut in one particular way. In each dish were planted 6 sclerotia or parts from 6 sclerotia. Sclerotia used in this experiment were stored in a granary all winter until used (March 3, 1956). Incubation was at 14° - 15° C. Final germination results were recorded May 8, 1956.

The final germination results for the sclerotial pieces as well as the whole sclerotia are given in table 12. From the data it is seen the sclerotial parts germinated just as readily as whole sclerotia. There is also a suggestion that the sclerotial peelings did not germinate as readily as the other cuttings or whole sclerotia. A later experiment showed a significant difference in the germination of sclerotial peelings from germination of other cuttings or whole sclerotia.

Table 12. Effect of sectioning on germination of sclerotia

Type of sectioning	No. of parts germinated / Total no. of parts per dish			% germination
	Dish 1	Dish 2	Dish 3	
Cross sections	19/22	10/36	8/24	45.12
Outer peelings	2/29	14/26	-*	29.09
Pillars of internal mycelium	3/6	2/6	-*	41.67
Longitudinal halves	1/12	5/12	6/12	33.33
Checks	4/6	3/6	0	38.89

* Contaminated sclerotial pieces discarded

Besides the stromata produced on the pillars of inner parts of the sclerotia, many were formed on the other cut surfaces (fig. 4 and 5). This was especially common in the case of the sclerotial peelings (fig. 6). Frequently stromata emerging from cut surfaces had very many vegetative hyphae about them. In some cases 4 - 5 heads emerged together from the cut surfaces. These cushion-like heads were pale and frequently covered by a soft mycelial weft. Stromata began to appear on all the different cuttings or the whole sclerotia at about the same time.

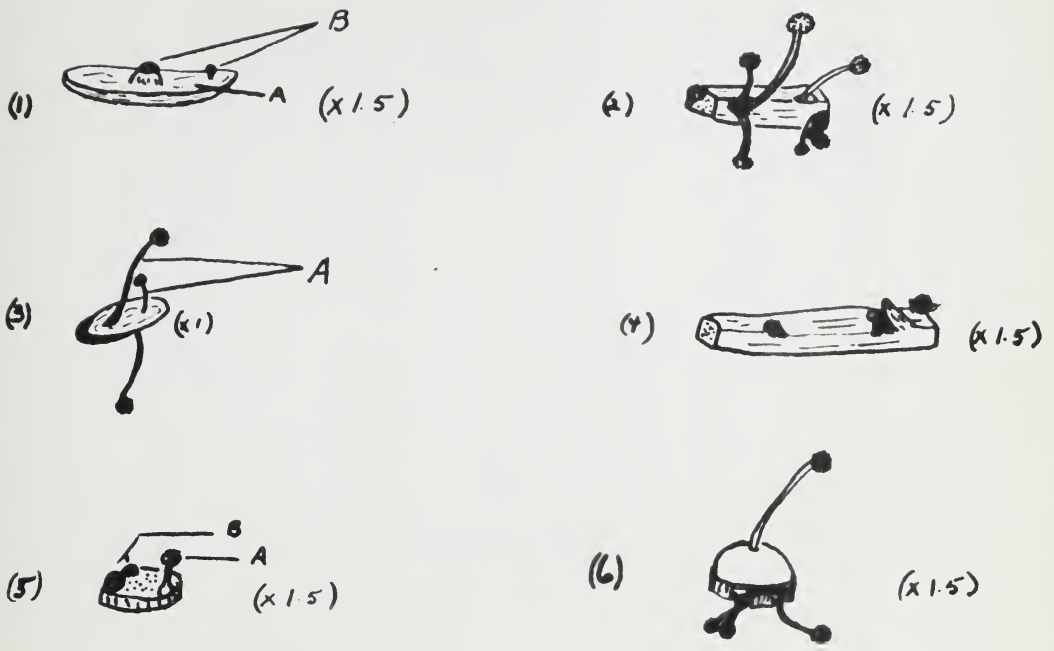

Fig. 4. Germination of sclerotial pieces.

(1) Half a sclerotium cut longitudinally; A. Cut surface; B. Stromata have developed on cut surface.
(2) Pillar of internal mycelium from sclerotium with normal, well-developed stromata.
(3) Peeling from sclerotium; A. Stromata have emerged at cut surface.
(4) Pillar of internal mycelium from sclerotium with stromata just emerging.
(5) Cross section of sclerotium; A. Stroma developed on the cut surface; B. Flesh-coloured cushion-like stromata.
(6) Cross section of sclerotium with well-developed stromata that emerged from intact margin.

Fig. 5. Cross sections of sclerotia with stromata that emerged from intact margins as well as cut surfaces.

Fig. 6. Peeling from sclerotium with mature and immature stromata.

The Germination of Sclerotia after Treatment with Growth Substances

Auxins have been found to affect the growth of many fungi. In many cases they are inhibitory even in relatively low concentrations or they give no response. Nysterakis (29), however, showed a definite growth response of a strain of Neurospora tetrasperma to indoleacetic acid (IAA). For many years it has been known that some fungi can synthesize IAA (10). Recently Gentile and Klein (11) found that Diplodia natalensis synthesizes IAA in just those concentrations required for close to optimal growth.

Barger (4) reported indications of tryptophan, a precursor of IAA, in sclerotia of C. purpurea. More recently Tyler and Schwarting (42) concluded from their experiments that indole is converted to tryptophan in C. purpurea.

Since a precursor of IAA is present in C. purpurea, it seemed profitable to determine what effect additions of various growth substances to the substratum would have on the germination of sclerotia. There was the possibility that the auxin might be at a suboptimal level in the sclerotia and additions would increase growth so that germination would be more rapid.

Experimental:

In the first experiments the ethyl ester of 2,4-dichlorophenoxyacetic acid (2,4-D) and IAA at concentrations of 5 p.p.m., 10 p.p.m., 20 p.p.m., 50 p.p.m., or 100 p.p.m. were used.

Sclerotia stored in a cooler (1° - 5° C.) or outside on the grass for 4 months were used in the first experiment. Sclerotia that were gathered in August, 1955 from a tetraploid and a diploid rye variety and overwintered in the granary were used in the second experiment. The auxins (water in the case of the checks) were used to moisten the greenhouse soil in the petri dishes. In each petri dish 5 sclerotia were planted. In the first experiment 4 dishes were used for each auxin concentration while 8 dishes were employed for each hormone treatment in the second test. The first experiment was started December 20, 1955 and final results were recorded February 20, 1956; the second experiment extended from March 1, 1956 to April 19, 1956. Incubation was at 14° - 15° C.

The results for the first and the second experiments are given in tables 13 and 14, respectively.

The data from table 13 show that the initial germination of the sclerotia used as a check in comparison with the germination of the sclerotia in the soil treated with different growth substances was much the same. However, the final results show that the sclerotia in soil treated with 5 p.p.m. 2,4-D and 20 p.p.m. 2,4-D germinated less frequently than the other sclerotia. The low germination of the sclerotia that received these two treatments was compared with the germination of the check by the t - test. The results were slightly greater than the one given for the 5% level. Therefore the differences in the germination of the treated

Table 13. Effect of two auxins at various concentrations on the germination of sclerotia

Auxin	Concentration p.p.m.	No. of sclerotia germinated per dish (5 planted)							
		Sclerotia stored in cooler				Sclerotia stored on grass			
		Dish 1		Dish 2		Dish 1		Dish 2	
		A*	B*	A	B	A	B	A	B
2,4-D	5	0	2	0	2	0	0	3	5
2,4-D	10	4	5	1	5	0	4	1	2
2,4-D	20	0	1	3	4	0	0	1	4
2,4-D	50	2	4	1	5	0	1	0	4
2,4-D	100	1	2	2	3	1	2	1	4
IAA	5	0	3	0	3	0	5	1	3
IAA	10	0	4	0	2	3	5	0	4
IAA	20	5	5	2	2	0	3	0	0
IAA	50	1	5	2	3	0	4	0	5
IAA	100	0	5	1	5	0	5	1	5
Check	-	0	5	1	5	0	5	1	5

* A: No. of sclerotia germinated January 5, 1956;
B: No. of sclerotia germinated February 20, 1956.

and untreated sclerotia may have been due to the variability of the sclerotia. The data also show that the manner in which the sclerotia were stored prior to germination had no effect on their germination.

Table 14. Effect of two auxins on the germination of sclerotia

Auxin	Concentration p.p.m.	No. of sclerotia germinated per dish (5 planted)													
		Sclerotia from diploid rye								Sclerotia from tetraploid rye					
		Dish 1		Dish 2		Dish 3		Dish 4		Dish 5		Dish 6		Dish 7		Dish 8	
		A*	B*	A	B	A	B	A	B	A	B	A	B	A	B	A	B
IAA	100	1	5	1	2	2	2	1	1	5	5	4	4	4	5	4	4
2,4-D	100	1	2	2	2	3	4	2	2	4	5	3	5	4	5	4	5
Check	-	4	5	5	5	3	4	3	4	4	5	5	5	5	5	5	5

* A: No. of sclerotia germinated April 7, 1956;
B: No. of sclerotia germinated April 19, 1956.

The data in table 14 show that the initial germination of sclerotia in soil given the auxin treatments was generally less than the germination of the sclerotia that served as a check. By a comparison of the initial results for each treatment with the check by the t - test, values slightly greater than those given for the 5% point were obtained. With such results it is not certain if there was any real difference. In any case the final germination of sclerotia planted in the soil moistened with auxin solutions was not significantly different from that of the sclerotia in soil dampened with water. The results also show there was no significant difference in the final germination of sclerotia from the two rye hosts. However, the initial germination of the sclerotia from the two rye varieties was significantly different (by t - test).

Lack of noteworthy differences in the germination of sclerotia in auxin-treated soil from the germination of sclerotia in the soil moistened with water may be attributed to the action of the soil on the auxins. Audus (3) stated that when an auxin is added to the soil some of it is taken up and retained by the soil colloidal particles. Greater quantities are leached away. With time the bacterial flora capable of removing the hormone is built up; the time required for this varies with the auxin employed (14 - 20 days for 2,4-D).

In order that the effects of the soil on the auxins might be eliminated sterile blotting paper in petri dishes served as the substratum in the next experiment. Indolebutryic acid (IBA), tryptophan, IAA, and 2,4-D at concentrations of 100 p.p.m., 250 p.p.m., and 500 p.p.m. were used. The sclerotia designated for one treatment were soaked for 90 min. in a particular auxin solution at one of the given concentrations. They were planted in petri dishes on blotting paper that was moistened with the same auxin solution. Other sclerotia, soaked for 90 min. in water and planted on water-dampened blotting paper in petri dishes, served as checks. Three dishes containing 6 sclerotia each were used for each treatment. Incubation was at 14° - 15° C. The sclerotia tested were from Prolific rye; storage was in a granary from September until April 17, 1956 when the experiment was started. Initial results were recorded May 17, 1956, while final results were taken June 15, 1956.

The germination data are given in table 15. By analysis of variance it was found that neither IAA, 2,4-D, nor tryptophan had any effect, at the concentrations used, on the time required for germination or on final germination of the sclerotia. Examination of table 15 shows the germination of sclerotia treated with IBA at 100 p.p.m. or 250 p.p.m. were not greatly different from the germination of sclerotia kept moist with sterile water.

Table 15. Effect of three auxins and tryptophan on germination of sclerotia

Chemical treatment	Concentration p.p.m.	No. of sclerotia germinated per dish (6 planted)					
		Dish 1		Dish 2		Dish 3	
		A*	B*	A	B	A	B
IAA	100	0	4	0	4	1	5
IAA	250	0	6	1	4	0	4
IAA	500	1	6	1	3	1	3
IBA	100	0	4	2	6	0	5
IBA	250	3	6	2	4	0	6
IBA**	500	-	-	-	-	-	-
2,4-D	100	2	4	0	2	0	1
2,4-D	250	0	6	2	5	0	3
2,4-D	500	1	1	0	5	0	3
Tryptophan	100	0	2	0	5	1	6
Tryptophan	250	0	3	1	4	3	3
Tryptophan	500	1	6	3	6	1	5
Check	-	1	3	2	5	3	4
Check	-	0	3	0	6	2	3
Check	-	1	2	2	4	1	5

* A: Initial results taken May 17, 1956;
B: Final results recorded June 15, 1956.

** Sclerotia discarded; damaged by water leakage from cooler.

The Effect of IAA Solutions of Different Concentrations on the Germination of Whole or Parts of Sclerotia

In the previous experiment the sclerotia were soaked for 90 min. in the different chemical solutions. While up-take of water by sclerotia in one hour is almost as great as the absorption of it in a week, it is probable that the absorption of the larger IAA molecules would not be as rapid. Therefore, in 90 min. the absorption of IAA by the sclerotia may not be very great. This consideration motivated a further experiment.

Experimental:

The sclerotia were soaked for 6 hr. in sterile, distilled water. Cross sections about 5 mm. in height, peelings about 1 mm. thick, longitudinal pillars of internal mycelium the length of the sclerotia and 2 - 3 mm. in diameter, and sclerotial halves were prepared as in a previous experiment. Whole sclerotia were also tested. The sclerotia or pieces of sclerotia were surface sterilized with 2% Javex solution for 3 min., washed with sterile, distilled water, and soaked in the IAA solutions (water in the case of the checks) for 17 hr. Six sclerotia or pieces from 6 sclerotia were put in each petri dish that contained blotting paper moistened with the appropriate IAA solution or water. The concentrations of IAA used were: 10 p.p.m., 50 p.p.m., 100 p.p.m. and 250 p.p.m. For each concentration of auxin with sections of one particular type 3 petri dishes were used. The sclerotia tested had been stored in a cooler (5° C.) from September 1, 1955 until May 30, 1956

when the experiment was started. The sclerotia or pieces were incubated at 14° - 15° C. Final results were recorded July 3, 1956.

Both rates of IAA used in the treatments and the manner of cutting the sclerotia were shown to be significant by the analysis of variance carried out on the data given in table 16. Just as it was found in a previous experiment, table 16 shows that sclerotial peelings germinated less well in comparison with whole sclerotia or cuttings of other kinds. The low values for the peelings were found to be significant when compared with the values for whole sclerotia by the t - test. A comparison of the final germination results for the pillars of internal mycelium and the whole sclerotia by the t - test showed a doubtful difference (t - value just slightly larger than the one at the 5% level). With cognizance of the results obtained in a previous experiment when sclerotial pieces were germinated, it is probable that the differences between the germination of the internal pillars and the whole sclerotia was due to variability of the sclerotia. The results also show that the sclerotia or parts treated with 250 p.p.m. IAA had a much lower germination than the sclerotia or parts treated with water only (significant difference found by t - test).

Table 16. Effect of various concentrations of IAA on germination of whole or parts of sclerotia

Manner of cutting	Concentration of IAA p.p.m.	No. of sclerotia or parts germinated per dish (6 planted)					
		Dish 1		Dish 2		Dish 3	
		A*	B*	A	B	A	B
Cross sections	0	3	6	2	2	1	1
Longitudinal halves	0	2	4	0	1	3	3
Pillars of internal mycelium	0	2	3	1	1	2	2
Peelings	0	0	1	0	0	0	0
(Whole sclerotia)	0	0	1	1	1	1	1
Cross sections	10	1	2	1	2	1	1
Longitudinal halves	10	0	0	2	2	2	2
Pillars of internal mycelium	10	2	2	4	4	1	3
Peelings	10	0	0	0	1	2	2
(Whole sclerotia)	10	1	1	0	0	2	2
Cross sections	50	0	0	0	0	0	0
Longitudinal halves	50	0	2	1	3	1	1
Pillars of internal mycelium	50	1	3	0	1	0	5
Peelings	50	0	1	0	0	0	0
(Whole sclerotia)	50	1	2	3	4	0	1
Cross sections	100	0	2	0	1	3	3
Longitudinal halves	100	0	3	3	5	1	2
Pillars of internal mycelium	100	2	4	3	5	1	1
Peelings	100	0	0	0	0	1	1
(Whole sclerotia)	100	0	0	1	3	1	2
Cross sections	250	0	0	0	0	0	0
Longitudinal halves	250	1	2	0	0	0	0
Pillars of internal mycelium	250	0	1	0	2	1	2
Peelings	250	0	0	0	0	0	0
(Whole sclerotia)	250	0	0	1	1	0	2

* A: Initial results recorded June 23, 1956
B: Final results recorded July 3, 1956.

To the longer soaking period along with the cutting up of the sclerotia must be attributed the significant results obtained for the 250 p.p.m. IAA treatment. In two experiments the sclerotial peelings have been shown less germinative than other cuttings or whole sclerotia. The very low results recorded for the peelings in the latter experiment may have been effected by the surface sterilization of the sclerotia with the 2% Javex solution. In a previous experiment on soaking of sclerotia it was shown that surface sterilization with Javex inhibited the germination. Sclerotial peelings have a relatively thin layer of germinative hyphae. It is quite possible that such a thin layer would be readily affected by the Javex solution.

The Effect of Phenolic Solutions on the Germination of Sclerotia

Experimental:

A preliminary experiment suggested that sclerotia treated with 1% phenol for 2 - 3 min. germinated better than sclerotia not treated. Sclerotia that had overwintered on the grass were surface sterilized with the phenolic solution and placed on sterile blotting paper in petri dishes. In each of 3 petri dishes were put 9 sclerotia. A similar number of untreated sclerotia in 3 dishes served as a check. Incubation was at 14° - 15° C. The experiment was started July 16, 1956 and final results were taken September 4, 1956.

The sclerotia surface sterilized with the phenol solution germinated sooner than the check. By August 22, 1956, 67% of the treated sclerotia and 6% of the untreated ones had germinated. Results taken September 4, 1956 showed 85% of the sclerotia surface sterilized with the phenol solution and 31% of the unsterilized ones germinated.

An experiment involving more sclerotia per treatment and more phenolic compounds was started October 4, 1956. One per cent solutions of phenol, o-cresol, and resorcinol were each used to surface sterilize 40 sclerotia for 2 min. The sclerotia were then washed in sterile, distilled water and placed on sterile blotting paper in petri dishes. Each dish contained 10 sclerotia. Incubation was at 12° C. The sclerotia tested were gathered in August, 1956 and were kept in a cooler (5° C.) until the experiment was started. Initial and final results were recorded December 7, 1956 and December 27, 1956, respectively.

Table 17. Effect of surface sterilization with phenolic compounds on germination of sclerotia

Treatment	No. of sclerotia germinated per dish (10 planted)							
	Dish 1		Dish 2		Dish 3		Dish 4	
	A*	B*	A	B	A	B	A	B
1% phenol	3	4	1	5	1	6	1	5
1% o-cresol	3	5	2	4	0	3	0	2
1% resorcinol	2	7	3	4	2	7	3	4
Check	1	5	0	5	1	6	1	7

* A: No. of sclerotia germinated December 7, 1956
B: No. of sclerotia germinated December 27, 1956.

The initial results as recorded in table 17 show that the sclerotia treated with resorcinol were slightly in advance of the others in germination. However, by the final results it is seen the germination of all sclerotia was much the same.

The stimulatory effect of the phenol surface sterilization in the first experiment was probably due to the bactericidal and fungicidal activity of phenol. The sclerotia that overwintered on the grass would be expected to have many microorganisms on their surfaces and in their numerous cracks.

The Effect of Certain Nutrients on the Germination of Sclerotia

The influence of nutrients on the mycelial growth and conidial production of C. purpurea in agar or liquid cultures has been investigated extensively. While Taber and Vining (40) did not find maltose readily utilized by the three strains of this fungus studied, McCrae(25) and Gjerstad and Ramstad (12) did note better growth of C. purpurea in a medium containing this sugar. Similarly, Robbins and Ma (34) found malt increased the growth of C. purpurea. It is generally agreed that asparagine is a good nitrogen source for this fungus. McCrae (25) found vitamin E stimulated the growth of C. purpurea. Unlike earlier investigators Taber and Vining (40) found an exogenous supply of biotin was required for their C. purpurea cultures.

Experimental:

Although sclerotia are well stocked with nitrogenous compounds and carbohydrates (4), it was decided to determine what

effect additions of certain nutrients would have on germination. The solutions used in the first experiment were: 2% maltose, 2% maltose and 0.03% asparagine, and 100 p.p.m. biotin. The solvent used was sterile, distilled water. An emulsion of wheat germ oil in water was prepared by mixing in a waring blendor 10 drops wheat germ oil in a 100 ml. sterile, distilled water. For 24 hr. 24 sclerotia were soaked in each solution. Each lot of sclerotia was then placed in petri dishes on sterile, blotting paper that was moistened with the nutrient solution having the same constituent(s) as the one in which they were soaked. Sclerotia, soaked for 24 hr. in sterile, distilled water and then placed in petri dishes that contained blotting paper moistened with sterile water, served as a check. Six sclerotia were planted in each dish. The sclerotia had been stored on the grass from September, 1955 until May, 1956. From May, 1956 until June 16, 1956, when the sclerotia were used, they were kept in a cooler (5° C.). Incubation was at 15° C. Final results were recorded July 25, 1956.

Germination occurred in all dishes at about the same time.

From table 18 it is seen that sclerotia treated with 2% maltose and 0.03% asparagine had the best germination. A comparison of the germination in 2% maltose and 0.03% asparagine with that in water by the t - test showed a significant difference.

Table 18. Effect of certain nutrients on germination of sclerotia

Treatment	No. of sclerotia germinated per dish (6 planted)			
	Dish 1	Dish 2	Dish 3	Dish 4
2% maltose	2	1	1	1
2% maltose, 0.03% asparagine	5	6	3	5
Wheat germ oil emulsion*	2	2	3	1
100 p.p.m. biotin	0	4	4	2
Check	1	3	0	1

* 1 drop of oil per 10 ml. H_2O

Another experiment was performed in a manner similar to the one just described. Using sterile, distilled water as the solvent the following solutions were prepared: 1000 p.p.m. asparagine, 1% maltose and 1000 p.p.m. asparagine, 1% maltose and 250 p.p.m. asparagine, and 100 p.p.m. biotin. In each solution 36 sclerotia were soaked for 15.5 hr. In each petri dish containing sterile blotting paper 9 sclerotia were placed. A check was prepared as in the previous experiment (soaking time 15.5 hr.). The sclerotia tested were collected in September, 1956 and stored in a cooler (5° C.) until used (November 3, 1956). Final results were recorded January 15, 1957.

Table 19 shows that germination after treatment with 1% maltose and 250 p.p.m. asparagine was better than after treatment with any of the other solutions or water. The difference in germination between the sclerotia treated in 1% maltose and 250 p.p.m.

Table 19. Effect of certain nutrients on germination of sclerotia

Treatment	No. of sclerotia germinated per dish (9 planted)			
	Dish 1	Dish 2	Dish 3	Dish 4
1% maltose, 1000 p.p.m. asparagine	4	8	5	4
1% maltose, 250 p.p.m. asparagine	7	8	5	7
1000 p.p.m. asparagine	5	4	4	6
100 p.p.m. biotin	3	5	6	2
Check	2	3	6	2

asparagine and the check was not found significant (t - test). Possibly the change in concentrations of the two substances and the time of soaking were not as favourable for germination as the ones used in the previous experiment.

The Effect of Various Common Fungicides on the Germination of Sclerotia

Brentzel (6) studied the effects of Ceresan, Arasan, Spergon, Basul, Cuprocide, copper carbonate and formaldehyde on the germination of sclerotia that were planted on soil. The sclerotia were treated at various rates from 0.5 to 8 oz. per 60 lb. sclerotia. While his results were very erratic, he concluded that Ceresan, Arasan, Spergon, Cuprocide and Basul were not effective in control.

Experimental:

The effect of Orthocide 75, Arasan, Spergon, Ceresan M, 5% commercial formaldehyde, and 1000 p.p.m. mercuric chloride on the germination of sclerotia was determined. The dry fungicides were placed in separate erlenmyer flasks along with 40 sclerotia, and the flasks were shaken until the sclerotia were thoroughly covered with the fungicides. The fungicides were used at the rate of 0.0625 gm. per gm. sclerotia; this is equivalent to 1 oz. per lb. sclerotia. The liquid treatments (including sterile, distilled water for the checks) were given for 5 min. The sclerotia were placed in sterile petri dishes (10 per dish) containing moist blotting paper. All dishes were placed in the cooler at 12° - 14° C. Sclerotia were collected in the latter part of August, 1956 and were stored in a cooler (5° C.) until used (November 13, 1956). Germination results were recorded January 26, 1957.

Table 20. Effect of some fungicides on germination of sclerotia on blotting paper

Fungicide	Concentration	No. of sclerotia germinated per dish (10 planted)			
		Dish 1	Dish 2	Dish 3	Dish 4
Orthocide 75	1 oz./lb. sclerotia	3	5	3	3
Spergon	1 oz./lb. sclerotia	4	2	2	7
Arasan	1 oz./lb. sclerotia	0	0	0	0
Ceresan M	1 oz./lb. sclerotia	0	0	0	0
Formaldehyde	5% commercial solution	1	0	3	0
Mercuric chloride	1000 p.p.m.	9	8	4	7
Check	-	9	1	7	10

The germination results are seen in table 20. Why Ceresan and Arasan treatments should have prevented germination of the sclerotia is not too clear. As stated previously, Brentzel (6) had good germination of sclerotia treated with these two fungicides in some of his experiments. Possibly the planting of the sclerotia on blotting paper rather than in soil was influential. (This is considered in more detail in a later discussion.) In comparing the other treatments only the 5% commercial formaldehyde solution gave values significantly different from the checks (by t - test).

The Effect of Streptomycin on the Germination of Sclerotia

Experimental:

The bactericide, streptomycin, was tested on sclerotia to see what effect it might have on the germination of sclerotia. By its ability to greatly reduce the bacterial flora on the sclerotia, it was believed it might possibly increase the germination.

Sclerotia that had been stored at 5° C. from September, 1956 until used (December 15, 1956) were planted in petri dishes that contained sterile blotting paper soaked with 100 p.p.m. streptomycin sulphate or sterile distilled water (in the case of the check). Four petri dishes containing 10 sclerotia each were used.

The results taken February 7, 1957 showed that the germination of the sclerotia moistened with 100 p.p.m. streptomycin sulphate was very much the same as for the ones moistened with

water. Percentage germination for the former ones was 56 while for the latter it was 55.

The Effect of Some Inorganic Chemicals on the Germination of Sclerotia

St. Garay (36) found that hydrogen peroxide at 5×10^{-3} M inhibited the germination of conidia. McCrae (25) found that hydrogen peroxide always blackened her cultures of C. purpurea no matter whether it was sprayed above them or in direct contact with them. Ammonium sulphate, an important fertilizer, was found by Hacskazlo et al (16) to be a poor source of nitrogen for 25 different fungi tested.

Experimental:

Sclerotia were soaked (40 per treatment) in freshly prepared 1% solutions of hydrogen peroxide, ammonium sulphate, and ammonium hydroxide for 30 min. Into each petri dish that contained sterile blotting paper were placed 10 sclerotia. The blotting paper was soaked with a solution having the same chemical make-up as the one used for the preliminary soaking. Another 80 sclerotia, (40 of them surface sterilized with 1000 p.p.m. mercuric chloride for 5 min.) were soaked for 30 min. in sterile, distilled water and placed in petri dishes on sterile blotting paper wetted with sterile, distilled water. All sclerotia tested were collected at the beginning of September from Prolific rye and stored in a cooler (5° C.) until October 19, 1956 when they were used. The results were recorded January 18, 1957.

Table 21. Effect of some inorganics on germination of sclerotia

Treatment	No. of sclerotia germinated per dish (10 planted)			
	Dish 1	Dish 2	Dish 3	Dish 4
1% H_2O_2	4	0	6	1
1% $(NH_4)_2SO_4$	0	0	0	0
1% NH_4OH	0	0	0	0
1000 p.p.m. $HgCl_2$*	1	5	5	2
Check	9	6	5	7

* Only surface sterilization for 5 min.

By table 21 it is seen that the best germination occurred in the check. Sclerotia treated with 1% hydrogen peroxide were still germinative. Possibly the ergothioneine found in sclerotia (4) prevented any inhibitory action (36). The ammonium hydroxide and ammonium sulphate solutions clearly inhibited the germination of sclerotia at the concentrations employed.

The Effect of Fungal Parasites on the Germination of Sclerotia

Langdon (23) has reported the growth of Cerebella spp. on the honey dew produced in flowers infected by C. purpurea. Development of C. purpurea was found to cease once the honey dew became infested with Cerebella spp. Therefore, Langdon concluded that Cerebella spp. aid in the natural control of ergot by inhibiting the development of the sclerotia.

The sclerotia of C. purpurea were observed in these studies to be overgrown under certain conditions by various fungi. Those used in the following experiment with the exception of Verticillium sp. were isolated from sclerotia during 1955 and 1956 at Edmonton. Trichothecium roseum, a fungus that grows readily on the sclerotia of C. purpurea, has been reported by Démétriades and Papaioannon (9) to have attacked and destroyed sclerotia of Sclerotinia sclerotiorum.

Experimental:

In order to determine the effect of different fungi on C. purpurea, Trichoderma sp., Gliocladium sp., Cladosporium sp., Stemphylium sp., Verticillium sp., and Trichothecium roseum were grown on potato dextrose agar in test tubes and then transferred to a cornmeal - sand medium. The preparation of this medium and the manner in which the fungi were grown on it and then transferred to sterile soil have been described. Before inoculation with the above fungi, 8 sclerotia were placed in each dish. Five dishes were used for each treatment. Sclerotia planted on sterile soil

served as a check. Sclerotia were stored in a cooler (5° C.) from September until January 10, 1957 when the experiment was started. Incubation was at 15° C. Final results were taken March 15, 1957.

Table 22. Effect of certain fungi on germination of sclerotia

Fungus	No. of sclerotia germinated per plate (8 planted)				
	Dish 1	Dish 2	Dish 3	Dish 4	Dish 5
Gliocladium sp.	0	0	0	0	0
Trichothecium roseum	0	0	0	0	3*
Trichoderma sp.	0	0	0	0	0
Cladosporium sp.	1	6	0**	0	0
Verticillium sp.	3	1	2	2	3
Stemphylium sp.	4	4	1	1	3
Check	4	4	2	2	1

* For some unknown reason there was no fungal growth on this plate
** Plate contaminated by other fungi.

By reference to table 22 it may be seen that Gliocladium sp., Trichothecium sp. and Trichoderma sp. prevented the germination of the sclerotia. These fungi completely covered the sclerotia within a month. The other fungi did not grow well on the sclerotia and so had little effect on their germination. In dish 5 Trichothecium roseum did not grow.

The Effect of Partial Destruction of Sclerotia by a Beetle on their Germination

Petch (30) reported that ergot bodies are often attacked and entirely consumed by insects.

During August, 1956, 3000 sclerotia were gathered from brome grass in a pasture and along the adjacent roadside near Brandon, Manitoba. Upon examining these sclerotia it was found that about half of them had a rough, powdery spot on one side. Within each such sclerotium was found the larva of an insect. Two weeks later the larvae in the sclerotia had developed into tiny black beetles. The beetle was identified as Acylomus sp.

On November 17, 1956 an experiment was started to determine what effect, if any, the growth of Acylomus sp. in the sclerotia had on their subsequent germination.

Experimental:

Insect-riddled sclerotia as well as an equal number of normal ones were removed from a cooler (5° C.) where they had been kept since August. After the growth of Acylomus sp. in the sclerotia, all that remained of many of them were small pieces. Therefore, many of the normal ergot bodies were broken into pieces comparable to the ones that had been partially devoured. All sclerotia or sclerotial pieces were planted on sterile blotting paper and placed in a cooler at 15° C. Two petri dishes, containing 24 sclerotia or pieces each, were used for the insect-riddled as well as the normal. The germination was the same for the former

as for the latter. Out of 48 sclerotial pieces used in each case 42% germinated. The germination was slow. Not until February were any stromata seen. The results were taken February 11, 1957.

The whole sclerotia that germinated never produced more than three stromata. This was very different from the majority of sclerotia germinated (fig. 2) that came from rye varieties rather than brome grass. Tiffany (41) found that the number of stromata produced depended on the host from which the sclerotia came.

Discussion

After reaching maturity, sclerotia enter a dormancy period. The length of this period depends very much on the conditions to which the sclerotia are exposed. Although Kirchoff (22) found that exposure of sclerotia to temperatures below or near zero for 3 - 6 weeks was essential for good germination, it has been shown that this is not always true. Sclerotia buried beneath a thin covering of black soil in the field during September and the first part of October, 1955 (about 9° C.) germinated well within 3 weeks at 15° - 18° C. While temperatures are not favourable for out-of-door germination in the late autumn in central Alberta, it is possible that sclerotia of C. purpurea could germinate at this time in regions having longer falls.

The fact that sclerotia did germinate on many substrata not known to contain any nutrients that this fungus will utilize suggests that only moisture is required for the germination of ergot bodies. For example, Henson and Valleau (19) found 1% water agar a good medium on which to germinate sclerotia of C. purpurea, and the present studies have shown that moist, sterile, blotting paper is just as good as various soils. Taber and Vining (40) found that the conidial stage of the fungus did not utilize cellulose as a carbon source.

Results obtained in this study suggest that grey soil may be more favourable for the germination of sclerotia of C. purpurea than black or brown. A larger experiment involving many

more sclerotia than have been used would be required to determine whether this soil is an especially good medium for the germination of sclerotia.

Soils with different microflorae are most certain to affect the germination of the sclerotia. In this connection it is significant that Gliocladium sp., found commonly on sclerotia planted in fresh, Edmonton, black soil or greenhouse soil, was able to prevent the germination of sclerotia. Trichoderma sp., isolated from sclerotia planted on fresh, brown soil from southern Alberta, was also effective in preventing the germination of sclerotia. It is quite possible that some substance(s) produced by one or both of these fungi (or from Trichothecium roseum which also inhibits the germination of ergot bodies) could be utilized to prevent the germination of the sclerotia.

Moisture content of the substratum and the temperature to which the sclerotia are exposed are two physical factors greatly influencing the germination of sclerotia. Significantly, the moisture content of the soil in which good germination occurred was fairly high: 23 - 24%. Markhasseva (26) also reported that a soil moisture content of 22% was best under natural conditions. In view of these two high similar results it seems reasonable to place some credence in reports (4) of a high correlation between wet springs and severe outbreaks of ergot in cereal crops. Another experiment on moisture of the substratum showed that ergot bodies germinated in sand with a moisture content that had a wide range. Since the sclerotia in nature would be expected to behave

in a similar manner, some of them would germinate in soil with a moisture content well below and above 23 - 24%. Henry (17) found some sclerotia would germinate in a soil with 10% moisture. Thus, the fungus is able to survive in relatively dry or wet seasons. Similarly, the wide range of temperatures at which sclerotia germinated (10° ± 2° C. to 23° ± 2° C.) indicates that the fungus can continue its life cycle during cold as well as relatively hot springs and early summers. Sclerotia that can germinate under such variable temperature and moisture conditions are well suited to survive under the vagaries of nature.

Soaking, as such, for a considerable period of time is not expected to inhibit seriously the germination of sclerotia. At least, sclerotia soaked for 4 days at 15° C. under nonsterile conditions germinated readily. Indeed, Démétriades and Papaioannon (9) have found that sclerotia of Sclerotinia sclerotiorum after submergence in sterile water for 8 months still retained their viability. The water soaking at 24° ± 2° C. for 89 hr. was probably inhibitory to the germination of sclerotia of C. purpurea because of the considerable bacterial growth in the water. Possibly some chemical(s) were excreted by the bacteria that adversely affected the sclerotia, or some food substance(s) stored in the sclerotia and necessary for germination was removed by the bacteria. In relation to the latter possibility it is recalled that the water-soaked, soft sclerotia, when planted on the greenhouse soil, were readily engulfed and rotted by numerous fungi and bacteria.

Why water-logged sclerotia after being frozen at -10° C. for one week had lower germination than the others is not clear. Certainly freezing sclerotia in moist, sandy soil (0° F. to -5° F.) as recommended by Brentzel (6) for good germination represents similar conditions as obtained in the above experiment, although less extreme with regard to moisture content of sclerotia. Even though the sclerotia were placed under conditions suitable for germination one week later than the others they were still given 8 weeks to germinate. The most probable explanation of the low germination of water soaked sclerotia is that freezing of them in such a condition delays their germination very greatly. In any case they are still germinative; two different lots gave the same germination (20%) on two different substrata.

Various chemicals were found to influence the germination of sclerotia. A 2% Javex solution (a hypochlorite) used in surface sterilization did lower the germination of sclerotia on a sand substratum. The decrease in germination probably resulted from the oxidation of some compound(s) in the sclerotia necessary for good germination. Germination of sclerotia was completely inhibited by 1% ammonium hydroxide and 1% ammonium sulphate. Hacskaylo et al. (16) believed the poor utilization of ammonium sulphate by the 25 fungi they studied was due to the pH of the ammonium sulphate solution. The ammonium hydroxide and ammonium sulphate solutions used had pH values of 10 and 5.5, respectively. According to experiments on the effects of pH values on the germination of sclerotia, an initial pH of 5.5 did not depress germination to an appreciable extent. While the high pH of the ammonium hydroxide solution was

probably influential in reducing sclerotial germination, the reduction of germination by ammonium sulphate cannot be explained in the same manner. In any case Taber and Vining (40) have shown that the ammonium radical does not harm the growth of the conidial stage of C. purpurea. It is of interest that in soil sanitation programs ammonium sulphate is often mentioned as a good chemical to prevent the development of certain fungi. That 250 p.p.m. IAA solution was found to inhibit the germination of sclerotial pieces was consistent with the effect of this auxin on saprophytic growth of the conidial stage of C. purpurea. In an experiment performed, 50 p.p.m. IAA in Leonian's medium did not affect the size of the colonies of C. purpurea. However, at 100 p.p.m. IAA concentration the growth was inhibited somewhat, while at 500 p.p.m. IAA no growth occurred in 3 dishes and in the fourth the colony was 0.25 the diameter of the checks.

Although further experiments are required, results to date have indicated that certain nitrogenous substances (e.g., asparagine) along with certain carbohydrates (e.g., maltose) did affect the germination of sclerotia at least at some concentrations. Conceivably sugars, such as glucose and fructose, and nitrogenous substances other than asparagine that are readily utilized by this fungus might decrease the time for germination and increase the percentage germination. Sclerotia are well-stocked with various nitrogenous substances and three carbohydrates - trehalose, mannitol, and clavicepsin - according to Barger (4), but neither sugars such as maltose, glucose, and fructose nor asparagine have been found.

Before reaching any general conclusion regarding the value of a particular fungicide in preventing germination of sclerotia in all substrata, it is necessary to remember that the substratum on which the treated sclerotia are planted must be considered. Rushdi and Jeffers (35) found that certain soil factors controlled fungicidal activity. These were soil texture, pH, and organic content. Therefore, the efficiency of Arasan and Ceresan M in preventing the germination of sclerotia of C. purpurea on moist blotting paper does not necessarily hold true for other substrata. This point is illustrated by Brentzel's work (6). Good germination of sclerotia treated with Arasan and Ceresan occurred in some of his tests when the substratum was a soil. By the same token, sclerotia that have germinated well on blotting paper would not necessarily germinate as well on another substratum after this treatment. Use of blotting paper rather than soil had the value of showing more truly the effect of the fungicide on the sclerotia. Since the sclerotia were lying upon the sterile blotting paper rather than embedded in it, there was very little loss of fungicide from the ergot bodies by adsorption. Rushdi and Jeffers (35) concluded that the degree of adsorption by different soils is the most important factor affecting activity of soil fungicides. Guillemat and Lelièvre (15) found that 1% commercial formalin on potato tubers covered with sclerotia of Corticium solani prevented the germination of the latter. Sclerotia of C. purpurea soaked for 5 min. in 5% commercial formalin did not germinate readily on a blotting paper substratum.

Normally, when a sclerotium germinates the outer rind is split for a short distance longitudinally, or a semi-circular flap of external hyphae is pushed up by the emerging head. Cross sections of sclerotia germinated in this manner show that stromata originate about 0.5 mm. below the surface. However, when sclerotial pieces were germinated it was found that the normal stromata did originate in a few instances in mycelium further from the surface than 0.5 mm. Longitudinal halves had stromata originating from the cut surface. Since stromata originated at all these levels, it is believed that all hyphae except the dark-walled, external ones are potential places of origin of stromata.

Even though sclerotia had been partially devoured by larvae of Acylomus sp. they still germinated. Unless the larva consumed almost all the sclerotium its attack would not be expected to prevent germination.

Sclerotia from a diploid rye variety did not germinate as rapidly as sclerotia from a tetraploid rye variety, even though they were gathered from ripe rye within a few days of one another and they were kept under the same storage conditions. It may be readily seen (and many times reported) that mature sclerotia from tetraploid rye and diploid rye differ in size. The sclerotia from tetraploid rye are on the average larger than those from diploid rye. Larger sclerotia would be expected to have a greater quantity of stored food as well as more hyphae potentially able to produce stromata. Possibly these two presumed differences in sclerotia were operative in influencing rapidity of germination.

III. Development of Stromata

Some Physical Factors Affecting the Development of the Stromata of C. purpurea

Two Tropic Movements of the Stromata

Grasso (13) found that the stromata of Claviceps spp. are positively phototropic, and Barger (4) stated they are negatively geotropic. Two experiments were performed to determine whether the stromata, produced by the sclerotia that were used for a germination test previously described, behave similarly.

Experimental:

In determining whether stromata are positively or negatively phototropic, sclerotia that had been germinated in a water soaking experiment in January, 1957 were used. Sclerotia were chosen whose stromata were 0.25 in. or less above the sclerotial surfaces. Into each sterile petri dish containing moist blotting paper were placed 6 germinated sclerotia. Two of the 4 dishes used were completely covered with thin black paper. The other 2 dishes had each an opening 2 in. x 1 in. in the black paper at one side of the dish. The 4 cultures were placed on a shelf in an incubator (61° - 62° F.). The dishes with openings in the black paper were turned so that unilateral light rays would enter them. At 4 ft. from the dishes was a 200 watt bulb enclosed in a thick transparent glass shield. The dishes received light continuously for 2 weeks.

By the end of 14 days the stromata that received light had developed well, but they were all bent towards the light.

The stromata in the dishes kept in the dark had grown in every direction. The lengths of the stipes were greater than in the former case. Figure 7 shows the difference in the development of the stromata in unilateral light from that in darkness.

Another experiment was set up in exactly the same way as the one just described. However, in this experiment all the stromatal heads were removed. The stromatal stipes that received unilateral light did not bend towards the light. This action is reminiscent of that of the oat coleoptile in unilateral light after its tip is excised. All of the stipes regenerated new heads. This aspect is discussed in more detail later.

The geotropic response of stromata was determined by using 5 sclerotia also from the soaking experiment. Each sclerotium whose stromata had emerged about 0.25 in. above the sclerotial surface was secured to the end of a piece of thread 4 in. in length. The other end of each thread was glued to a glass plate. The plate was placed over a glass dish (5 in. deep) so that the germinated sclerotia hung freely in the dish. The dish was kept moist by thoroughly soaking with distilled water a thick layer of blotting paper placed in the bottom of it. Dish and glass covering were completely surrounded by black paper so that sclerotia were kept in the dark. The wrapped dish was placed in an incubator (61° - 62° F.) on February 2, 1957.

The dish was opened February 20, 1957. All stromata were found well developed and growing in a general upward direction. That is, the stromata were negatively geotropic.

The Effect of Temperature on the Growth and Development of Stromata

On a synthetic agar medium McCrae(25) found the mycelial growth of C. purpurea was directly proportional to the temperature increase over a range from 10° C. to about 30° C. No comparable controlled experiments have been described concerning the effect of temperature on the growth and development of the stromata of C. purpurea. Therefore, an experiment designed to give some information on this question was performed.

Experimental:

Some of the sclerotia that were germinated in the water soaking experiment carried out during January, 1957 were tested. Into each of 9 petri dishes containing sterile, moist blotting paper were placed 6 sclerotia whose stromata were 0.5 cm. or less above the sclerotial surface. All dishes were wrapped in thin black paper. Three dishes were placed at each of the following temperatures: 12° C., 17.5° C., and 24° - 27° C. Incubation at these temperatures was continued for 13 days. At the end of that time the lengths of all the stromata were determined. The average stromatal length for each sclerotium was calculated.

In table 23 the average length of the stromata per sclerotium grown at a particular temperature is given. By use of the t - test a significant difference was found between the average lengths of the stromata raised at 12° C. and the ones grown at 17.5° C. That is, the stromata at 12° C. did not grow linearly as

Table 23. Effect of temperature on linear growth of stromata

Temperature (° C.)	Average length (cm.) of stromata for each sclerotium																	
12°	0.34	0.90	0.88	1.02	0.72	0.76	0.10	0.65	0.37	0.40	0.97	1.03	0.50	0.83	0.40	0.57	0.73	0.38
17.5°	1.97	0.84	1.17	2.39	1.52	1.79	1.53	1.13	0.73	2.16	1.03	1.15	1.65	2.30	1.30	2.19	2.06	1.23
24° - 27°	0.95	2.08	2.02	0.10	1.30	1.54	2.55	1.50	2.45	1.05	1.30	0.86	0.80	1.34	1.72	0.10	0.40	1.44

well as the ones at 17.5° C. No such significant difference was found between the stromata raised at 17.5° C. and those grown at 24° - 27° C.

After the incubation period of 13 days mature ascospores were found in the asci developed at all the temperatures.

The diameters of the stromatal heads developed at 17.5° C. ranged from 1 mm. to 4 mm. while those formed at the other two temperatures ranged from 1 mm. to 2 mm. Similarly the diameters of the stipes of the stromata developed at 17.5° C. ranged from 0.5 mm. to 3 mm. and the ones developed at the other temperatures were from 0.5 mm. to 1.5 mm. Many of the stromata that developed at 24° - 27° C. had pale violet stipes and light yellow heads; stromata formed at 12° C. and 17.5° C. had purple to brown stipes and reddish-brown heads.

The Effect of Light Intensity on the Growth and Development of the Stromata

Purdy (31) found that for the germination of the sclerotia of Sclerotinia sclerotiorum no light was necessary, but light was needed for the normal development and expansion of the apothecial disks. Accordingly, an experiment was performed to see what effect different intensities of light would have on the growth and development of the stromata of C. purpurea.

Experimental:

Sclerotia germinated in the phenolic surface sterilization test were employed in this experiment. The stromata on the sclerotia

chosen were just emerging. Into each sterile petri dish containing moist blotting paper were placed 7 sclerotia. Six dishes were prepared. All dishes were put in the same cooler at 61° - 62° F. where temperature variation from shelf to shelf was negligible. On one shelf 4 ft. from a 200 watt bulb were placed 4 plates; two of these were wrapped in black paper. The other remaining plates were set on the shelf below so that they were in the shadow of the first shelf. The dishes remained under these different light intensities for 16 days. At the end of that time all lengths of the stromata were recorded. The average length of the stromata for each sclerotium was calculated.

By referring to table 24 it is seen from the average lengths of the stromata that the ones kept in bright light did not grow as well as the others confined to diffused light or darkness. A comparison of the average lengths of the stromata raised in darkness with the average lengths of the ones grown in bright light showed there was a significant difference (t - test). But, no such difference existed between the stromata confined to darkness and the others exposed to diffused light.

Mature ascospores developed underall the lighting conditions employed.

The Effect of Moisture in the Substratum on the Growth and Development of the Stromata

It was shown in a previous experiment that the percentage moisture content of the substratum for good germination of sclerotia had a relatively wide range (table 4). Therefore, a white sand

Table 24. Effect of light intensity on linear growth of stromata

Nature of light	Average length (cm.) of stromata for each sclerotium													
Bright light	1.12	0.90	0.40	0.50	0.80	0.20	0.40	0.25	0.20	0.39	0.57	0.55	0.83	0.40
Diffused light	2.41	1.53	0.20	0.60	0.42	0.80	0.36	0.63	1.37	0.90	1.92	1.06	0.78	1.63
No light	1.53	1.41	0.65	0.32	2.25	0.57	0.38	1.30	1.54	1.68	1.74	1.64	1.25	0.42

substratum with a moisture range was used for germinated sclerotia to determine what effect it would have on growth and development of the stromata.

Experimental:

Into each of 3 petri dishes was placed 50 gm. washed and dried white sand. Different quantities of sterile, distilled water were added to each dish before 5 germinated sclerotia (previously serving as a check in an experiment testing the effect of inorganics on germination) were planted in each one. The dishes were then weighed and placed in a cooler (15° - 16° C.) for 14 days. The dishes were weighed periodically and sterile, distilled water was added when necessary to bring the weightsback to the original ones. At the end of 14 days the stromata were removed from the sclerotia and measured. The average length of the stromata per sclerotium was calculated. The percentage moisture in each dish of sand was found in exactly the same manner as in the experiment on the effect of moisture content of the substratum on the germination of sclerotia (p. 21). All sclerotia tested had been subjected to a moisture condition suitable for good germination.

The average lengths of the stromata that were grown in the sand having a given moisture content are shown in table 25. While the average lengths of the stromata raised in a substratum having 14.74% moisture (by wt.) were found to be less than the average lengths for other two they were not significantly less (t - test). Of the three moisture percentages 10.88% gave the best stromatal growth.

Mature ascospores were developed under all three moisture conditions.

Table 25. Effect of moisture in substratum on linear growth of stromata

% moisture of sand (by wt.)	Average length (cm.) of stromata for each sclerotium				
4.58	1.44	2.25	0.46	0.52	1.10
10.88	3.25	0.58	0.50	1.40	1.93
14.74	2.05	1.30	0.44	1.02	0.13

Regeneration of Stromata on Excised Stipes

Grasso (13) discussed various incidents of regeneration of beheaded stromata of Claviceps spp. A description of the regeneration of the capitula of C. paspali was given by him in detail. It was found that after the heads were cut off the wounded parts of the stalks became covered after a few days with a pale, loose mycelium that later became ochraceous and compact. This gradually developed into a normal appearing capitulum. In a few weeks after the perithecia appeared mature, the new capitulum grew away from the scar on the original stipe by means of a short peduncle.

Numerous experiments were carried out using sclerotia of C. purpurea that had developed mature or nearly mature stromata to determine whether new capitula on the excised stipes would

Fig. 7. Stromata are positively phototropic. Stromata at left received unilateral light; stromata at right were in darkness.

Fig. 8. Regeneration of stromata at tops of excised stipes, X 5.

develop as they did for C. paspali. Other interesting aspects concerning regeneration of capitula were also studied.

Experimental:

The heads of well developed, intact stromata were removed, or the stipes were cut off about halfway from the bases to the heads. In other cases the mature stromata were cut off at their bases. The sclerotia with the basal stromatal stumps were saved as well as the stromata. These stromata also had their heads removed. In practically all tests excised parts were placed on sterile blotting paper moistened with sterile, distilled water. However, a few sclerotia with excised stipes were planted in moist, greenhouse soil in petri dishes. The dishes were incubated at temperatures from 15° to 18° C.

Development of secondary stromata occurred in practically all instances regardless of the substratum on which the sclerotia were placed. The development of new stromata on the excised stipes was essentially the same for C. purpurea as Grasso described for C. paspali. The time taken for new stromata to appear was generally 12 - 16 days, when the stipes were still attached to the sclerotia, but it was much longer when they were removed from the latter. The new stromata were not always produced at the cut surface, as Grasso found for C. paspali. Frequently development was halfway up to the stipe or at the base of the stipe (fig. 9 and 10). For every stipe cut there was frequently an umbel of 3 - 5 new stromata produced at the apex (fig. 8), part way up the stipe, or at the

base. At other times the secondary stromata were produced uniseriately along the stipes (fig. 10). When secondary stromata occurred part way up the stipes they formed from the loose mycelium that sometimes developed along the exterior (fig. 10 and 11) or they formed in the interior of the stipe. In the latter case, at the region of their emergence the primary stipe was split into longitudinal ribbons (fig. 9). The secondary stipes (Grasso called them peduncles.) grew just as long as the primary ones (fig. 9).

Stipes cut just part way up from the bottom, rather than just below the heads, regenerated new stromata in a manner similar to the others.

Stipes removed from the sclerotia seldomly developed secondary stromata. Whenever they did so, 1 - 2 months were required. Development of the stromata in this case occurred only at the bases of the cut stipes or part way up them (fig. 9).

Sclerotia with all the stromata removed regenerated new ones. The new stromata generally occurred in clumps about and on the old stromatal stumps.

Mature ascospores were produced in the secondary heads only after the secondary stromata had well developed stipes. Such a developmental stage was never reached by secondary stromata developed on stipes removed from the sclerotia.

Secondary stromata developed just as rapidly in bright light (4 ft. away from 200 watt bulb) as in darkness or diffused light.

After mature secondary stromata, produced at the apex of the primary stipes, were beheaded, tertiary ones appeared at the bases of the secondary ones (i.e., at the scar on the primary stipe) after 3 months time (fig. 10). Tertiary stromata never developed to maturity.

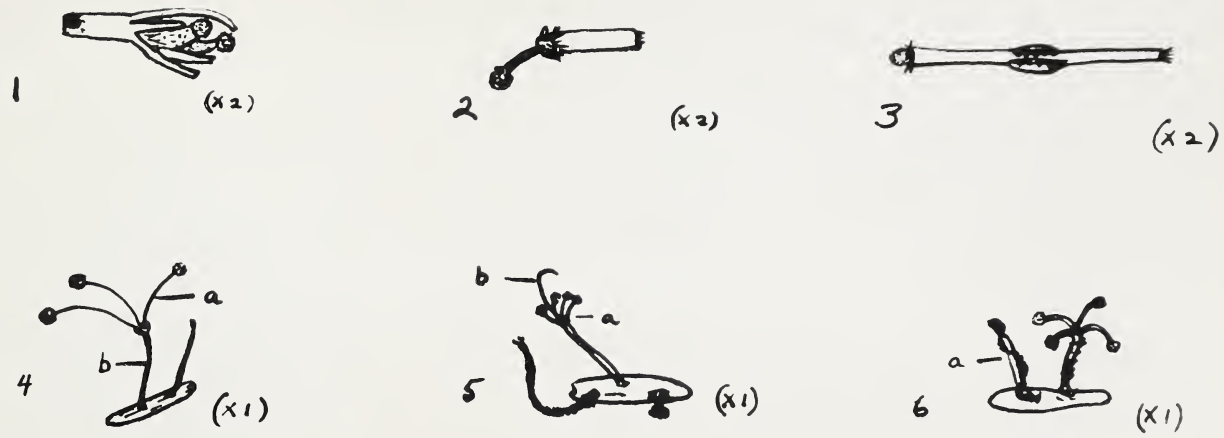

Fig. 9. Regeneration of stromata on excised stipes.

(1) Secondary stromata developed within stipe cut from sclerotium.
(2) Secondary stroma formed at basal end of stipe.
(3) Secondary stroma forming at base of stipe and others developing within stipe.
(4) Well developed secondary stromata at top of excised stipe; a - secondary stroma; b - primary excised stipe.
(5) Secondary stromata developed part way up the excised stipe; a - umbel of secondary stromata; b - upper part of primary, excised stipe.
(6) Regeneration of stromata at tip of excised stipe; a - mycelial growth in spiral band on stipe.

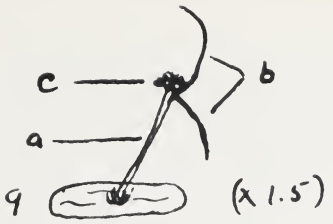

Fig. 10. Regeneration of stromata on excised stipes. (continued)

(7) Secondary stromata developed uniseriately along excised stipes.
(8) Umbel of secondary stromata at base of excised stipe.
(9) Tertiary stromata forming at bases of secondary stipes;
a - primary stipe; b - secondary stipes; c - tertiary stromata.

Fig. 11. Regeneration of stromata on excised stipes, X 5.

Discussion

That the stromata of C. purpurea were positively phototropic and negatively geotropic suggests that an auxin is operative in stromatal growth, as IAA is in higher plants.

The best temperature for stromatal development was 17.5° C., of the three temperatures employed. The linear growth of stromata at 17.5° C. was not significantly different from that at 24° - 27° C., but it was significantly different from that at 12° C. Therefore the temperature range for the best growth of stromata appears to be higher than that for best germination of sclerotia (10° ± 2° C. to 20° ± 2° C.).

Bright light definitely decreased the linear growth of stromata. This also suggests that a light sensitive auxin is operative in stromatal growth. In any case the growth processes for the stromata of C. purpurea must be different from the ones for the Ascomycete Sclerotinia sclerotiorum which requires light for good growth and development of apothecia (31).

Stipes completely removed from sclerotia regenerated new stromata. So, the ability to produce stromata must remain in the primary stipes. However, stipes wholly removed from sclerotia did not produce new stromata readily, nor did the stromata that were produced reach maturity. Therefore, it would seem that food substances stored in the sclerotia are essential for good development of secondary stromata. That considerable reserve food was still

present in sclerotia that produced mature stromata was seen by their ability to produce secondary stromata after 3 - 4 weeks at 15° C. Since this second "crop" of stromata originated about the bases of the primary stipes, in most cases, it is believed that the hyphae at the bases of the stipes were more readily able to divide and grow than other hyphae in the sclerotia.

IV. GENERAL DISCUSSION

Studies on certain environmental factors have shown that C. purpurea sclerotia are admirably well adapted to carry the fungus over from one growing season until the next in the temperate zones. Although it was found that an average temperature of 9° C. was able to break the dormancy of sclerotia, when they were outside covered by a thin layer of soil, considerably lower temperatures (6) are recommended for breaking the dormancy period. Such temperatures prevail during our winter season. Very many substrata, as long as they were moist, were found suitable for sclerotial germination. The relatively wide moisture range and temperature range under which the sclerotia germinated indicate the ability of this fungus to produce the perfect stage of its life cycle under a great variety of climatic conditions that may occur in late spring and early summer.

A number of practical ways of breaking the life cycle of C. purpurea at the sclerotial stage or the post sclerotial stage have been suggested by results of experiments reported here. Burying sclerotia by deep ploughing would prevent the dissemination of ascospores because the stromatal heads were only able to reach the surface in two cases out of approximately 1500 when planted beneath soil two inches deep. Since sclerotia failed to germinate when planted on blotting paper moistened with a 1% solution of ammonium hydroxide or ammonium sulphate, it may be found that the use of one or the other of these fertilizers at a practical concentration will decrease the sclerotial germination. Since

surface sterilization of sclerotia with 2% Javex for 3 min. did decrease the germination of sclerotia when they were planted on sand, such a treatment of infested seed before planting in soil might have some value in decreasing the sclerotial germination. The great reduction in the number of sclerotia that germinated after a soaking for 89 hr. in sterile, distilled water at 24° ± 2° C. suggests a possible value of flooding sclerotia-infested soil to decrease the germination of the ergot bodies. Moore (28) has shown that flooding the soil will control Sclerotinia sclerotiorum in Florida by rotting the sclerotia.

Spraying crops with 2,4-D is not likely to affect the germination of the sclerotia in the soil beneath the grain. Concentrations of 500 p.p.m. 2,4-D did not affect the germination of the sclerotia on blotting paper. It is unknown what the approximate concentration of 2,4-D would be in the soil after a spraying, but it would not be very high because the grain leaves and weeds would receive most of the liquid. That which would reach the soil would be diluted by the moisture there and by the following rains. In addition the adsorption of the auxin by the soil particles, the leaching of it by the rain, and the inactivation by certain bacteria would further decrease the 2,4-D concentration (3).

Some results have indicated the difficulty of preventing the sclerotia from germinating and developing mature stromata. Even peelings from sclerotia 1 mm. thick, cross sections 3-5 mm. in height, and inner longitudinal pillars of mycelium as well as sclerotial halves germinated and produced normal stromata, when the pieces

were planted on moist blotting paper or moist sand. When each stromatal head was removed usually 3 - 5 new stromata were formed at the apex of the stipe, along the stipe, or at its base. In the capitula mature ascospores were produced. Sclerotia that had produced mature stromata produced a second "crop" after the first ones were removed.

The germination of sclerotial pieces has a practical aspect. Often when grain is threshed the sclerotia are broken into pieces so small they are difficult to remove. Since the sclerotia are broken, there will be more units in the seed. This will mean a wider distribution of the pathogen in a form that may withstand wide variations in the environment and still produce the primary inoculum (ascospores) when favourable conditions prevail.

V. SUMMARY

1. Experiments were performed to determine the effects of certain physical, chemical, and biological factors on the germination of the sclerotia of Claviceps purpurea (Fries) Tulasne. The effect of some physical factors on the growth and development of the stromata was also studied.

2. Sclerotia required a dormant period before germination. This dormancy was broken by allowing the sclerotia to remain outside beneath a thin covering of soil at about 9° C. for 5 weeks in the autumn in which the sclerotia matured. Less than three weeks at 15° - 18° C. were then necessary for germination of the sclerotia in greenhouse soil.

3. Sclerotia were germinated in a number of different Alberta and B.C. soil types, varying in pH and other characteristics. They were also successfully germinated on white sand and on blotting paper.

4. Initial pH values near neutrality appeared to favour the germination of the sclerotia.

5. Sclerotia germinated in white sand with a moisture content as low as 1.43% (by wt.) and as high as 16.12% (by wt.). In black soil 100% germination occurred when the moisture content was 23 - 24% (by wt.).

6. At temperatures from 10° ± 2° C. to 23° ± 2° C. sclerotia germinated.

7. Sclerotia soaked for 89 hr. in sterile, distilled water at 3° - 4° C. or at 17° C. germinated readily while those soaked in the same manner at 24° ± 2° C. germinated poorly. Soaking sclerotia for four days at 15° C. and then freezing at -10° C. for a week decreased the germination of the sclerotia.

8. Neither one exposure of sclerotia to ultra-violet rays for 10 min. (4.3 amp., 150 volts, 21 cm.) nor six exposures of 10 min. per week visibly affected the germination of the sclerotia or the development of the stromata.

9. There was significantly less germination of sclerotia planted on the surface of the soil than of sclerotia planted just beneath the surface, 2 in. below, or 6 in. below.

10. The alternate drying and wetting of the sclerotia for one month did not affect the germination time nor percentage germination.

11. Cross sections of sclerotia 0.3 - 0.5 mm. in height, peelings about 1 mm. thick, longitudinal pillars of internal mycelium, and longitudinal sclerotial halves all germinated.

12. Various auxins at concentrations up to 500 p.p.m. did not affect the germination of whole sclerotia when there was no preliminary soaking or a soaking for 90 min. Sclerotial pieces soaked in 250 p.p.m. IAA for 17 hr. and then planted on blotting paper moistened with this solution had significantly decreased germination.

13. Certain nutrient solutions increased the germination of sclerotia. A solution containing 2% maltose and 0.03% asparagine was the most effective.

14. Surface sterilization with 1% solutions of phenol, o-cresol or resorcinol for 2 min. did not affect the germination of sclerotia, but surface sterilization with 2% Javex solution for 3 - 4 min. did decrease the germination.

15. Sclerotia dusted with Orthocide 75 or Spergon at the rate of 1 oz. per lb. germinated readily, as did sclerotia treated with 1000 p.p.m. mercuric chloride for 5 min. Treatment with Arasan or Ceresan M at the same concentrations as the other dusts prevented the germination of sclerotia, while a 5% solution of commercial formalin employed for 5 min. decreased the germination of sclerotia. The sclerotia were planted on moist, blotting paper for these tests.

16. Treatment with 100 p.p.m. streptomycin sulphate did not affect the germination of sclerotia.

17. Germination of sclerotia was prevented by 1% solutions of ammonium hydroxide or ammonium sulphate; 1% hydrogen peroxide did not affect the germination of the sclerotia.

18. Certain fungi, namely Gliocladium sp., Trichoderma sp., and Trichothecium roseum, prevented the germination of sclerotia while others, such as Cladosporium sp., Stemphylium sp., and Verticillium sp., did not.

19. Sclerotia partially devoured by the larvae of Acylomus sp. were still germinative.

20. Stromata were found to be positively phototropic and negatively geotropic.

21. Bright light decreased the linear growth of stromata.

22. At temperatures of 24° - 27° C., and 17.5° C. the linear growth of stromata was about the same while the growth at 12° C. was significantly less. Of the three temperatures best development occurred at 17.5° C.

23. The linear growth of stromata was about the same when the germinated sclerotia were planted in white sand with the following moisture contents: 4.58%, 10.88% and 14.75% (by wt.).

24. Stipes removed from sclerotia produced secondary stromata after considerable time, but the stromata did not reach maturity. Beheaded stromata still attached to the sclerotia produced secondary stromata at the tips of the stipes, along the stipes, or at the bases of the stipes. Sclerotia that had produced mature stromata were able to produce a second "crop" of stromata after the first ones were cut off at their bases. Regeneration of stromata occurred in the darkness as well as in the bright light of a 200 watt bulb 4 ft. away.

VI. LITERATURE CITED

1. Anonymous. Life Magazine. Time Inc., Chicago, Ill. September 10, 1951.

2. Atanasoff, D. Ergot of grains and grasses. Stencilled and distributed by Office of Cereal Investigations, Bur. of Pl. Ind., U.S.D.A. 1920.

3. Audus, L.J. Plant Growth Substances. Leonard Hill Ltd., 9 Eden St., N.W. 1. 1953.

4. Barger, G. Ergot and Ergotism. Gurney & Jackson, London. 1931.

5. Békésy, N. Ein beitrag zur biologie mutterkorns. Phytopath. Z. 26, 1:49-56, 1956. (Abstr.). Rev. Applied Mycol. 35:521, 1956.

6. Brentzel, W.E. Studies on ergot of grains and grasses. Agr. Expt. Sta., N.D. Agr. Coll., Fargo, N.D. Bull. 348, 1947.

7. Brown, A.M. Abnormal colour in sclerotia of ergot, Claviceps purpurea (Fries) Tul. (Abstr.). Proc. Can. Phytopath. Soc. 14:14, 1946.

8. Campbell, W.P. Infectivity of cereal hosts by ergot from roadside grasses. (Abstr.). Proc. Can. Phytopath. Soc. 23:14-15, 1955.

9. Demétriades, S.D. and Papaioannon, A.J. Études sur la biologie du Sclerotinia sclerotiorum (Lib.) Massee. La formation des apothécies sous les conditions de l'Attique (Grèce). Ann. Inst. phytopath. Benaki 7, 2:95-111, 1953. (Abstr.). Rev. Applied Mycol. 34:803-804. 1955.

10. Dolk, H.E. and Thimann, K.V. Studies on the growth hormone of plants. Proc. Natl. Acad. Sci. (U.S.) 18:30-46, 1932.

11. Gentile, A.C. and Klein, R.M. The apparent necessity of indoleacetic acid for the growth of Diplodia (Fungi Imperfecti). Physiol. Plantarum 8:291-299, 1955.

12. Gjerstad, G. and Ramstad, E. Influence of certain chemicals on the growth and alkaloid formation of C. purpurea (Fries) Tulasne in artificial culture. J. Am. Pharm. Assoc. 44:736-740, 1955.

13. Grasso, V. Le Claviceps delle Graminacee italiane, Parti IV e V. Ann. Sper. Agr., N.S. 6:1507-1553, 1952.

14. Gröger, D. Qualitatszüchtung am mutterkorn, Kulturpflanze (Ber. Inst. Kulturpfl. Forsch) Supple. 1:226-238, 1956. (Abstr.). Rev. Applied Mycol. 35:762, 1956.

15. Guillemat, J. and Lelièvre, D. Étude de l'efficacité de quelques fongicides sur la vitalité des sclérotes du rhizoctone de la Pomme de terre. Pomme d. Terre franc. 1953, 161:16-24, 1953. (Abstr.). Rev. Applied Mycol. 34:394, 1956.

16. Hacskazlo, J. Lilly, V.G. and Barnett, H.L. Growth of fungi on three sources of nitrogen. Mycologia 46:691-701, 1954.

17. Henry, A.W. Natl. Res. Council Rev. Ottawa, Can. N.R.C. No. 2254: 223, 1950.

18. Henry, A.W., Gilpatrick, J.D. and Peterson, E.A. On the use of seed infested with the ergot fungus. (Abstr.). Proc. Can. Phytopath. Soc. 23:16, 1955.

19. Henson, L. and Valleau, W.D. The production of apothecia of Sclerotinia sclerotiorum and S. trifoliorum in culture. Phytopathology 30:869-873, 1940.

20. Killian, M.C. Sur la sexualité de l'ergot de siegle le Claviceps purpurea (Tulasne). Bull. de la Soc. Mycol. de France 35:182-197, 1917.

21. King, C.J. and Loomis, H.F. Studies on sclerotia and mycelial strands of the cotton-rot fungus. J. Agr. Research 42: 827-840, 1931.

22. Kirchoff, H. Beiträge zur biologie und physiologie des mutterkornpilzes. Centralbl. für Bakt., Ab. 2, 77: 310-369, 1929. (Abstr.). Rev. Applied Mycol. 8:560-562. 1929.

23. Langdon, R.F. The genus Cerebella Cesati - its biologic status and use. Phytopathology 32:613-617, 1942.

24. MacFarland, F.T. Factors affecting the germination of sclerotia of Claviceps (Ergot of rye). Science 56:85, 1922.

25. McCrae, A. The reactions of Claviceps purpurea to variations of environment. Am. J. Botany 18:50-78, 1931.

26. Markhasseva, V.A. A method for the prognosis of the development of ergot (Claviceps purpurea Tul.). Summ. Sci. Res. Wk. Inst. Pl. Prot. Leningr. 1935:535-537, 1936. (Abstr.). Rev. Applied Mycol. 16:31-32, 1937.

27. Miller, J.H. A revision of the classification of the Ascomycetes with special emphasis on the pyrenomycetes. Mycologia 41:99-127, 1949.

28. Moore, W.D. Flooding as a means of destroying the sclerotia of Sclerotinia sclerotiorum. Phytopathology 39:920-927, 1949.

29. Nysterakis, R. Resistence de Neurospora tetrasperma aux fortes doses d'auxine et relation avec l'hypothese de l'existence des»metabolites antiauxins». Compt. rend. 238:143-145, 1954.

30. Petch, T. More about Claviceps. Naturalist, London 1937: 25-28, 1937. (Abstr.). Rev. Applied Mycol. 17:104-105, 1938.

31. Purdy, L.H. Factors affecting apothecial production by Sclerotinia sclerotiorum. Phytopathology 46:409-410, 1956.

32. Ramstad, E. and Gjerstad, G. The parasitic growth of Claviceps purpurea (Fries) Tulasne on rye and its relation to alkaloid formation. J. Am. Pharm. Assoc., Sci. Ed. 44:741-743, 1955.

33. Remsberg, R.E. Studies in the genus Typhula. Mycologia 32:52-96, 1940.

34. Robbins, W.J. and Ma, R. Vitamin deficiencies of twelve fungi. Arch. Biochem. 1:219-229, 1942.

35. Rushdi, M. and Jeffers, W.F. Effect of some soil factors on efficiency of fungicides in controlling Rhizoctonia solani. Phytopathology 46:88-90, 1956.

36. St. Garay, A. Role of ergothioneine and catalase in infection by ergot fungus (Claviceps purpurea Tul.). Nature 177:91-92, 1956.

37. Schweizer, G. Über die kultur von Claviceps purpurea (Tul.) auf kaltsterilisierten nährböden. Phytopath. Z. 13, 4:317-350, 1941. (Abstr.). Rev. Applied Mycol. 21:70-71, 1942.

38. Seymour, E.K. and McFarland, F.T. Loss from rye ergot. Phytopathology (Abstr.). 11:41, 1921.

39. Stevens, F.L. Effects of ultra-violet radiation on various fungi. Botan. Gaz. 86:210-225, 1928.

40. Taber, W.A. and Vining, L.C. A nutritional study of three strains of Claviceps purpurea (Fr.) Tul. Can. J. Microbiol. 3:1-12, 1957.

41. Tiffany, L.H. Germination of sclerotia of Claviceps purpurea. Proc. Iowa Acad. Sci. 55:217-221, 1948.

42. Tyler, Jr., V.E. and Schwarting, A.E. The culture of Claviceps purpurea. III. Tryptophan metabolism. J. Am. Pharm. Assoc. 43:207-211, 1954.

43. Whetzel, H.H. and Reddick, D. A method of developing Claviceps. Phytopathology 1:50-52, 1911.

44. Youngken, Jr., H.W. Botany and Medicine. Am. J. Botany 43: 862-869, 1956.